The Prevalence, Clinical Features and Genetics of Epidermolysis Bullosa in Scotland

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Submitted for the degree of Doctor of Medicine

University of Edinburgh

2003

Abstract

Between May 1992 and June 2001, 309 epidermolysis bullosa (EB) sufferers were identified in Scotland (EB simplex [EBS] 175, dystrophic EB [DEB] 130, junctional EB [JEB] 4). The point prevalences of EB per million in June 2001 were: EBS (all variants) 33.2, the Dowling-Meara subtype of EBS (EBS-DM) 1.2, JEB 0.3, DEB (all subtypes) 24.6, dominant DEB (DDEB)17.4, DEB of uncertain inheritance (DEB-unc) 4.6, the Hallopeau-Siemens subtype of recessively inherited DEB (RDEB-HS) 1.4, localised RDEB (RDEB-loc) 1.0, and the inverse pattern of RDEB (RDEB-inv) 0.2. Incidences per million live births were EBS (all subtypes) 34.4, JEB 3.2, and DEB (all subtypes) 26.4. Although the prevalence figures for EBS and DEB are the highest of any yet reported, extrapolation of accurate data for the Lothians suggests that the prevalences of EBS and DEB in Scotland are underestimates.

Detailed clinical information on 130 EBS patients revealed considerable overlap between EBS-WC and EBS-Kb. As both phenotypes were frequently seen within the same pedigree and in patients bearing identical mutations, EBS-WC is best regarded as a mild variant of EBS-Kb rather than as a separate disorder. Improvement with age was common in EBS but not invariable. Nail involvement and aplasia cutis congenita were seen in all subtypes of EBS. Seasonal variation was confirmed as being common in EBS-Kb/EBS-WC and absent in EBS-DM. In contrast to previously held views, substantial minorities of those with EBS-Kb/EBS-WC experienced oral blisters (17%) and blisters at sites in addition to the palms and soles (43%). Oral blisters were previously thought to occur only in EBS-DM. Laryngeal involvement, usually associated with JEB, also occurred in EBS-DM.

Clinical features of DEB were recorded in 97 patients. Scarring, gastrointestinal involvement, albopapuloid lesions and EB-pruriginosa each occurred in both DDEB and RDEB. External ear involvement, a feature not often reported in DEB, was common in RDEB and also occurred in a minority of those with DDEB. With increasing age, squamous cell carcinoma was a major cause of mortality. Most of those with DDEB had relatively mild disease, their QOL scores reflecting less impairment of QOL than EBS sufferers. Whilst patients with RDEB-HS reported a greater impairment of QOL than patients with any other skin disease, EBS caused more pain than DDEB and had a greater effect on friendships, physical and non-leisure activities.

Twenty different keratin gene mutations (15 novel) were found amongst EBS patients, increasing the number of reported pathogenic keratin mutations by 23%. Disease severity was determined by the position of the mutation, but was also influenced by the nature of the amino acid substitution. Failure to detect keratin mutations in two families with classical EBS, suggests that abnormalities of genes other than KRT5 and KRT14 may cause an identical phenotype in a minority. In DDEB, 3 glycine substitution mutations of COL7A1, 2 novel, were found in 5 families. G2043R was confirmed as being a common cause of DDEB. Two novel mutations were found in patients with RDEB-HS, but identification of mutations in DEB patients proved difficult. The reasons for marked inter and intra familial phenotypic variation in both EBS and DEB are unknown.

Declaration of authenticity and acknowledgements

I confirm that this thesis is my own work and composition and that the research it is based on was undertaken entirely whilst I was in post in South-East Scotland. It has not been submitted in candididature for any other diploma, degree or professional qualification.

Information regarding mutations in epidermolysis bullosa patients was derived from laboratory work carried out by others on material sent to them by me. Extraction of DNA from epidermolysis bullosa simplex patients was kindly undertaken in the laboratories of Dr. D. Baty at Ninewells Hospital in Dundee. Subsequent mutation screening and sequencing of the genes encoding keratins 5 and 14 were carried out at the University of Dundee under the supervision of Professor E. Birgit Lane.

DNA from dystrophic and junctional epidermolysis bullosa patients was extracted and sequenced in the laboratories of Professor John McGrath at the St. Johns Institute for Dermatology in London.

I am grateful to the Dystrophic Epidermolysis Research Bullosa Association (UK) who funded this work, to Dr. E Rugg for her critical appraisal of Chapter Five and to Professor Andrew Finlay for permission to use the dermatology life quality questionnaires. I would also like to thank those clinicians who provided details of patients under their care and the many patients who took part in the study, remembering particularly those who have died.

To be involved in research into epidermolysis bullosa during an era when understanding of these disorders has advanced at an unprecedented rate has been a privilege which I owe directly to Dr. M. J. Tidman. I would like to thank him for his introduction to the subject and also for his un-failing patience and invaluable constructive criticism.

Finally, I am glad to acknowledge my husband and daughters, whose encouragement, understanding and support have sustained me.

Abbreviations

(C)DLQI	(children's) dermatology life quality index
COL7A1	gene encoding collagen VII
COL17A1	gene encoding collagen XVII
EB	epidermolysis bullosa
EBS	epidermolysis bullosa simplex
EBS-DM	Dowling-Meara subtype of epidermolysis bullosa simplex
EBS-Kb	Köbner subtype of epidermolysis bullosa simplex
EBS-MD	epidermolysis bullosa simplex with muscular dystrophy
EBS-MP	epidermolysis bullosa simplex with mottled pigmentation
EBS-WC	Weber-Cockayne subtype of epidermolysis bullosa simplex
DDEB	dominant dystrophic epidermolysis bullosa
DEB	dystrophic epidermolysis bullosa
DEBRA	Dystrophic Epidermolysis Bullosa Research Association
DEB-unc	dystrophic epidermolysis bullosa of uncertain inheritance
DLQI	dermatology life quality index
GABEB	generalised atrophic benign epidermolysis bullosa
ITGA6	gene encoding α6 polypeptide chain of integrin α6β4
ITGB4	gene encoding β4 polypeptide chain of integrin α6β4
JEB	junctional epidermolysis bullosa
JEB-H	Herlitz subtype of junctional epidermolysis bullosa
JEB-nH	non-Herlitz subtype of junctional epidermolysis bullosa
JEB-PA	junctional epidermolysis bullosa with pyloric atresia
K5	keratin 5
K14	keratin 14
KRT5	gene encoding keratin 5
KRT14	gene encoding keratin 14
LAMA3	gene encoding α3 polypeptide chain of laminin 5
LAMB3	gene encoding β3 polypeptide chain of laminin 5
LAMC2	gene encoding γ2 polypeptide chain of laminin 5
PLEC1	gene encoding plectin
QOL	quality of life
RDEB	recessive dystrophic epidermolysis bullosa
RDEB-HS	Hallopeau-Siemens variant of recessive dystrophic epidermolysis bullosa
RDEB-inv	recessive dystrophic epidermolysis bullosa - inverse pattern
RDEB-nHS	non-Hallopeau-Siemens variant of recessive dystrophic epidermolysis bullosa
RDEB-loc	localised recessive dystrophic epidermolysis bullosa
RIE	Royal Infirmary of Edinburgh
SCC	squamous cell carcinoma

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The Genetic Code

	Second base				
First Base	**U**	**C**	**A**	**G**	**Third base**
U	UUU Phe UUC " UUA Leu UUG "	UCU Ser UCC " UCA " UCG "	UAU Tyr UAC " UAA **Stop** UAG **Stop**	UGU Cys UGC " UGA **Stop** UGG Trp	**U** **C** **A** **G**
C	CUU Leu CUC " CUA " CUG "	CCU Pro CCC " CCA " CCG "	CAU His CAC " CAA Gln CAG "	CGU Arg CGC " CGA " CGG "	**U** **C** **A** **G**
A	AUU Ile AUC " AUA " *AUG Met	ACU Thr ACC " ACA " ACG "	AAU Asn AAC " AAA Lys AAG "	AGU Ser AGC " AGAArg AGG "	**U** **C** **A** **G**
G	GUU Val GUC " GUA " GUG "	GCU Ala GCC " GCA " GCG "	GAU Asp GAC " GAA Glu GAG "	GGU Gly GGC " GGA " GGG "	**U** **C** **A** **G**

Abbreviations for amino acids (short code for amino acid):

Ala	Alanine	(A)	Leu	Leucine	(L)
Arg	Arginine	(R)	Lys	Lysine	(K)
Asn	Asparagine	(N)	Met	Methionine	(M)
Asp	Aspartic acid	(D)	Phe	Phenylalanine	(F)
Cys	Cysteine	(C)	Pro	Proline	(P)
Gln	Glutamine	(Q)	Ser	Serine	(S)
Glu	Glutamic acid	(E)	Thr	Threonine	(T)
Gly	Glycine	(G)	Trp	Tryptophan	(W)
His	Histidine	(H)	Tyr	Tyrosine	(Y)
Ile	Isoleucine	(I)	Val	Valine	(V)

Stop = stop codon

* = start codon

Chapter One

Introduction

1.1. Definitions

The collective term "epidermolysis bullosa" (EB) refers to a group of inherited disorders in which blistering of the skin and mucous membranes occurs in response to minor mechanical trauma. Three major types of EB are recognized (Fine 2000), each defined according to the level of the cleavage plane at the dermoepidermal junction. In EB simplex (EBS), cleavage occurs through the subnuclear portion of basal keratinocytes; in junctional EB (JEB) the split is either through the lamina lucida or, rarely, through the lowermost part of basal keratinocytes (Mellerio and Pulkkinen 1998); in dystrophic EB (DEB), cleavage always occurs just below the lamina densa (Figure 1).

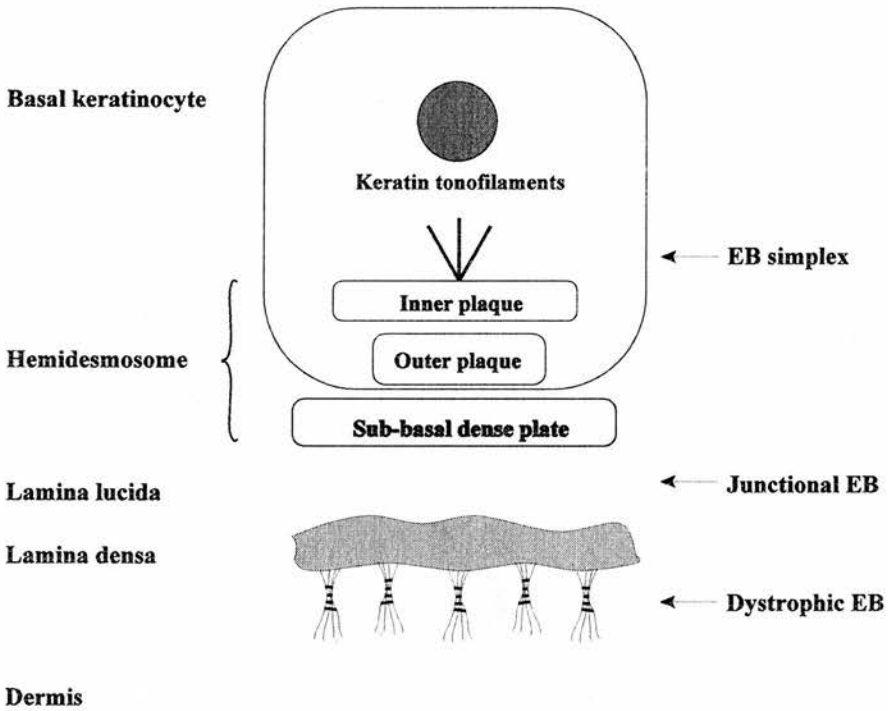
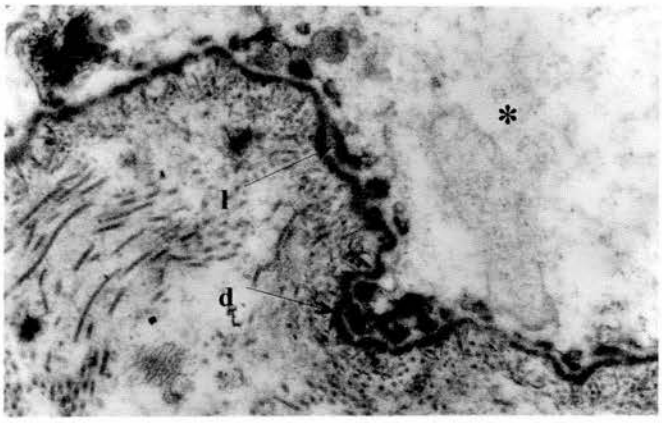
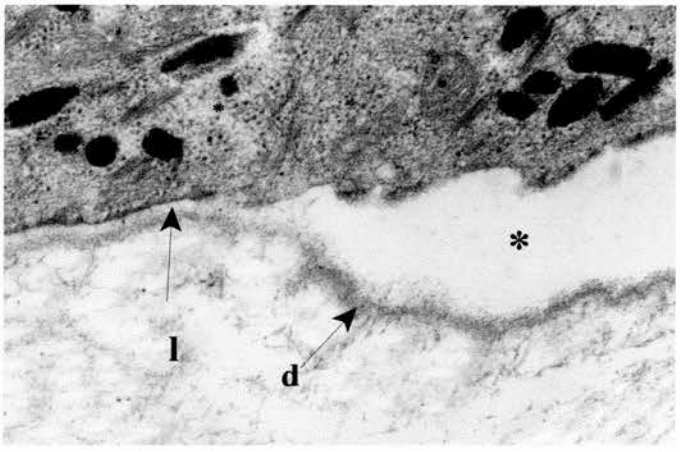


Figure 1. The dermoepidermal junction and EB.

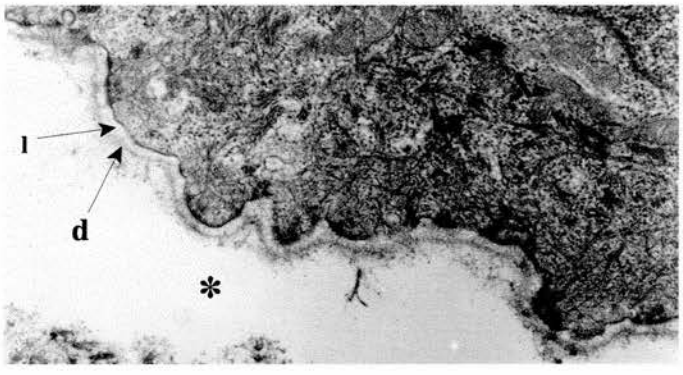
Figure 2. Ultrastructure of the dermoepidermal junction in EB.



A. EB simplex



B. Junctional EB



C. Dystrophic EB

* = blister cavity, d = lamina densa, l = lamina lucida

(Magnifications : A = x 18,000; B = x 28,000; C = x 15,000)

1.2. EB simplex.

EB was regarded as a variant of pemphigus (von Hebra 1870, Fox 1879) until Valentin (1885) used the term "hereditäre dermatitis bullosa" to describe what we would now recognize as EBS. The following year, for the first time, Köbner (1886) introduced the phrase "epidermolysis bullosa hereditaria" when reporting another family with the same disorder. The patients described all experienced dominantly inherited seasonal non-scarring blisters which occurred predominantly on the palms and soles during the warmer months (Figure 3). As both Valentin and Köbner pointed out, blisters also occurred at sites of friction from clothing, especially under "garters, braces and corsets". Blisters in similarly affected patients were observed on light microscopy to occur within the epidermis (Siemens 1921). A more localised variant of EBS in which blisters were confined to the feet, was described by both Weber (1926) and Cockayne (1938) under the title "recurrent bullous eruption of the feet". Both authors regarded this as an atypical variant of EB. It has since become customary to use the term "EBS Weber-Cockayne" (EBS-WC) to indicate localised EBS, in which only the palms and soles are involved and to refer to EBS- Köbner (EBS-Kb) when describing more widespread blistering. Some authors have preferred to reserve the title "EBS-Kb" for rare recessively inherited EBS with severe widespread blistering. In the literature there has been a tendency to use these terms interchangeably, sometimes without an accompanying clear clinical description . There remains confusion as to whether EBS-WC and EBS-Kb are identical conditions.

Dowling and Meara (1954) described four unrelated children who developed non-

seasonal spontaneous or trauma-induced blisters which were present at birth or appeared during the next 72 hours. Blisters were numerous, appearing on any body site including mucous membranes. Scarring was not a feature. The clinical picture resembled "juvenile dermatitis herpetiformis", blisters healing at the centre but spreading peripherally to produce "a circinate pattern of bullae" (Figure 4). The eldest child also had hyperkeratosis of the palms and soles. This disorder was recognized by Dowling and Meara as a form of EB, but they were unable to place it into the existing classification. Light microscopy of skin from their patients appeared to show subepidermal bullae, but more recently, electron microscopy of skin from similarly affected patients has shown that the split occurs through the subnuclear portion of basal keratinocytes (Anton-Lamprecht 1979), confirming this to be a variant of EBS. It is now well recognized and known as EBS-Dowling Meara (EBS-DM). In the neonate, blistering can occasionally be so extensive as to be fatal, but if this early period is survived, there is usually some improvement of the blistering tendency during childhood and adolescence and a normal life expectancy (McGrath 1992). Most patients have no preceding family history, but if more than one generation is affected, inheritance is autosomal dominant.

In all subtypes of EBS, the ultrastructural appearance is of a subnuclear split through basal keratinocytes and the hemidesmosomes appear normal. In EBS-DM, there is characteristic clumping of keratin tonofilaments which is not usually seen in other variants of EBS (Figure 5).

Figure 3. Blisters on the sole in EBS WC/Kb.

Figure 4. Herpetiform blisters of EBS-DM.

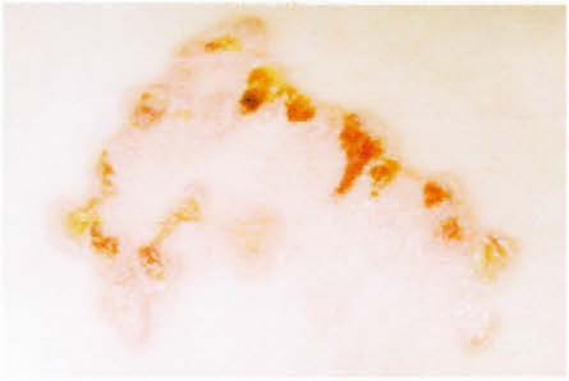
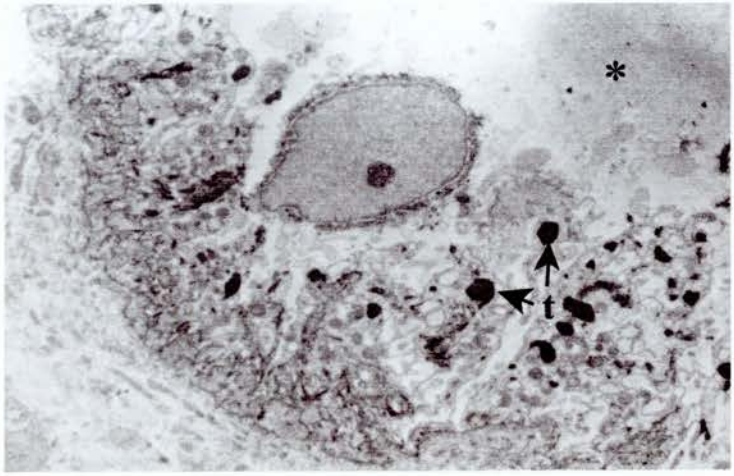


Figure 5. Tonofilament clumping within a suprabasal keratinocyte in EBS-DM.



* = blister cavity, t = clumped tonofilaments

(Original magnification:3,600)

1.3. Molecular basis of EBS

During the last decade, spectacular progress has been made in elucidating the causes of EB and it is now well established that EBS-WC, EBS-Kb and EBS-DM are due to mutations of genes on chromosomes 12 or 17 encoding keratin 5 (KRT5) or keratin 14 (KRT14) (Bonifas, Lane 1992). Both keratins are expressed within basal keratinocytes, forming heterodimers which are assembled into intermediate filaments. These form a dense network, acting as a cytoskeleton which imparts strength and rigidity to the cell. Keratin tonofilaments also provide cell to cell interconnections via desmosomes and attach keratinocytes to the basement membrane via hemidesmosomes (Figure 6) (Uitto 1997).

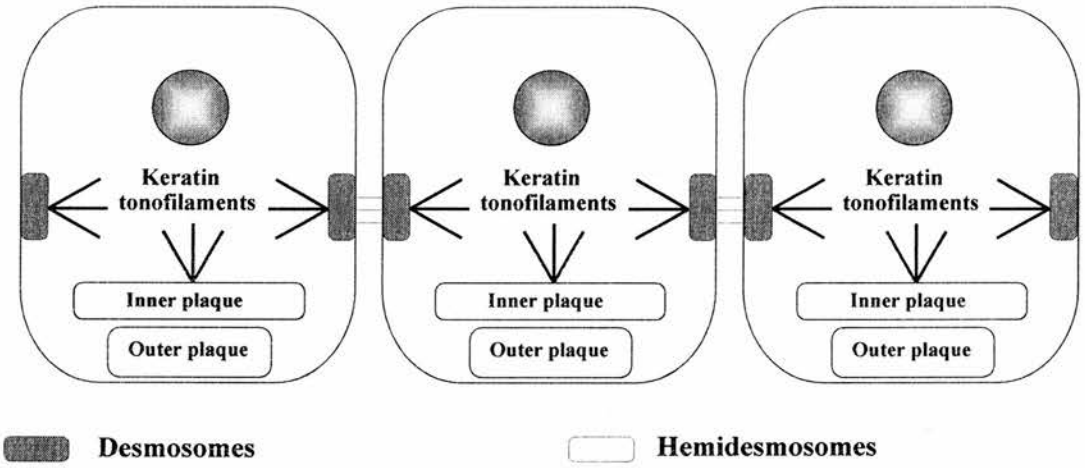


Figure 6. Attachment of keratin tonofilaments to desmosomes and hemidesmosomes

Both the site and nature of keratin gene mutations influence the phenotype. EBS-DM, the most severe subtype of EBS, is due to mutations occurring within the highly conserved helix initiation or termination motifs. Milder forms of EBS are seen when mutations occur at other sites (Irvine 1999).

Less common variants of EBS.

Other variants of EBS have been described more recently, but appear to be less common than the Weber-Cockayne and Dowling-Meara variants.

1.4. EBS with mottled pigmentation.

EBS with mottled pigmentation (EBS-MP), also inherited in dominant fashion, was first identified by Fischer and Gedde-Dahl (1979) and a total of eight unrelated affected families have now been reported. (Irvine 2001). Patients have non-scarring seasonal blisters occurring predominantly on the extremities and mottled macular pigmentation on the trunk and limbs. They also develop punctate keratoses of the palms and soles and most have nail abnormalities. The histological and ultrastructural appearances are similar to EBS-WC, EBS-Kb and EBS-DM, showing a sub-nuclear cleavage plane through basal keratinocytes and clumping of keratin tonofilaments. In seven of the eight families an identical causative point mutation of keratin 5 (P25L) has been reported (Irvine 2001).

1.5. Recessively inherited subtypes of EBS.

A small number of patients with homozygous mutations of keratin 14 have been reported (Hovnanian 1993, Rugg 1994, Chan 1994, Jonkman 1996, Corden 1998, Batta

2000). Most have severe widespread blistering but although Batta's patient was shown to have a complete absence of functional keratin 14, blistering in this patient was relatively mild. Recessive inheritance of EBS has to date been reported only in consanguineous families. In one large family, there is a clear dominant pattern of inheritance in most individuals, associated with mild disease. The more severely affected daughter of first cousins in this pedigree was shown to be homozygous for the same keratin 14 mutation responsible for mild blistering in each of her heterozygous parents (Hu 1997).

1.6. Plectin

1.6a EBS with muscular dystrophy.

EBS associated with muscular dystrophy (EBS-MD) is a rare autosomal recessive variant of EBS of widely varying severity (Niemi 1988, Shimizu and Takizawa 1999). Generalised blisters are usually present at birth or shortly thereafter. Occasionally blistering may be seasonal and atrophic scarring sometimes occurs. There is usually nail involvement and teeth are often abnormal. Some patients develop urethral strictures or scarring alopecia and a few infants have experienced respiratory difficulty (Mellerio 1997). Extensive cerebral and cerebellar atrophy has also been identified in some patients (Smith 1996). The age of onset of muscle weakness ranges widely from as early as 1 year to as late as 35 years. Mutations of varying disruptive potential have been described in these patients in the gene encoding plectin (Smith 1996, Shimizu and Takizawa 1999). Plectin is expressed in a wide variety of tissues, including skin, muscle and the central nervous system where it is found in association with the membrane

attachment sites of a variety of intermediate filaments. Within keratinocytes, it is localised to the inner plaques of hemidesmosomes (Figure 7) (Smith 1996, Pulkkinen and Uitto1999.) The cleavage plane in EBS-MD lies closer to the basement membrane than in other variants of EBS and hemidesmosomes appear abnormal. Like other subtypes of EB, the nature of the underlying mutations influences the severity of the phenotype (Shimizu, Takizawa et al 1999).

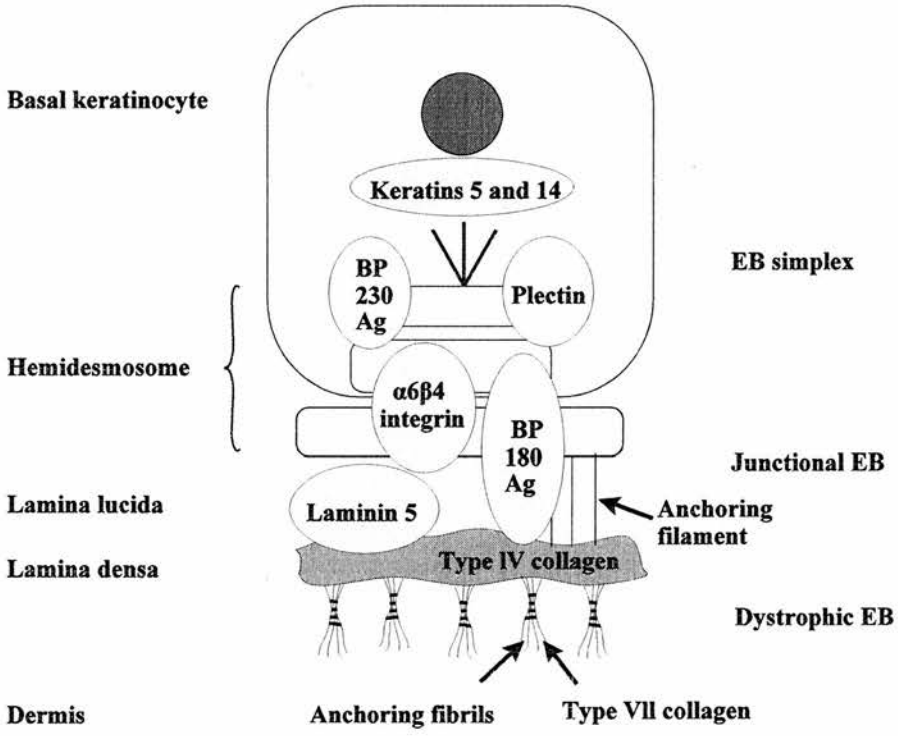


Figure 7. Localisation of the major components of the dermoepidermal junction.

Because the clinical features of EBS-MD vary so much, it is likely that many patients with this subtype of EBS have been mis-classified, especially those with minimally disruptive mutations or patients in whom weakness has yet to develop.

1.6b. Ogna variant of EBS

This variant of EBS was originally described in a single Norwegian family (Gedde-Dahl 1970). In addition to seasonal blistering of the palms and soles, sufferers had generalised non-seasonal skin fragility. This resulted in the occurrence of small erosions of a few days duration, on the extremities, limbs, face and occasionally on the trunk. Healing occurred without subsequent scarring. Many affected members of this large family also had dystrophic great toe nails. A second similar German family has since been identified and in both, inheritance is autosomal dominant. Recently, both families have been shown to carry an identical dominantly inherited mutation of plectin (Koss-Harnes 2002). In contrast to patients having recessively inherited mutations of plectin, muscle weakness has not occurred in any affected individuals in these two families.

1.7. Dystrophic EB.

Scarring variants of EB with clinical features distinct from EBS were described by Tilbury Fox (1879), Brocq (1890), Herzfeld (1892) and Hallopeau (1898). Both Cockayne (1933) and Touraine (1942) described larger clinical series of such patients with identical clinical features and who clearly had dominantly inherited disease. Patients experienced blisters particularly over their knees, elbows, hands and feet which left atrophic scars as they healed. Nails were missing or thickened and milia were common, especially in children (Figure 8). Patients with these clinical features would now be classified as having dominantly inherited DEB (DDEB) but mildly affected patients who showed only subtle scarring and minimal nail dystrophy were until quite recently often considered to have EBS. Pasini (1928) described two patients, who in

addition to the clinical features of DDEB, also had numerous small ivory-white papules on the trunk and limbs. He termed these "albo-papuloid lesions" (Figure 9). DDEB was traditionally sub-divided into two types - one in which albopapuloid lesions were present, termed Pasini-DEB, and a second, Cockayne-Touraine DEB, in which albopapuloid lesions were absent and which was thought to be less severe. The current classification recognises that both these variants of DDEB can occur within an individual pedigree and recommends that the distinction between them should be abandoned (Fine 2000). The cause and significance of albopapuloid lesions is unknown. They consist of collagen bundles and amorphous material within the upper dermis, the latter staining positively with Alcian blue (Nomura 1997).

Another group of patients show similar but more severe clinical features which Siemens (1921) pointed out were associated with recessive inheritance. Blisters are more widespread than in DDEB and are present from birth or the succeeding few hours. Like the more mildly affected patients, these individuals also have nail loss or dystrophy and develop scarring at sites of previous blisters. In addition, they develop anaemia, flexion contractures of fingers and limbs, oesophageal and urethral strictures and anal stenosis. There is extensive involvement of the oral cavity and scarring here causes restriction of mouth opening (microstomia) and tethering of the tongue (ankyloglossia). Scalp and body hair is sparse and affected individuals do not usually undergo puberty. A characteristic feature of this variant of recessively inherited DEB (RDEB) is the mitten deformity of the hands and feet (Figure 10).

Figure 8. Scarring, nail dystrophy and milia on the hand in DEB.

Figure 9. Albopapuloid lesions on the trunk in DEB.

Figure 10. Mitten deformity in RDEB-HS.

Webs of scar tissue form between adjacent digits, which become increasingly flexed and ultimately encased within a covering of skin. Infants and young children are at risk of developing septicaemia secondary to loss of extensive areas of skin, whilst 50% of those surviving to adult life develop at least one squamous cell carcinoma by the age of 40 (Uitto 2000). These tumours behave particularly aggressively in patients suffering from the Hallopeau-Siemens variant of RDEB (RDEB-HS), most dying within 5 years of detection of their first tumour (Uitto 2000). Not all patients with recessively inherited DEB have such a severe phenotype, some having a localized form of the disease and others having predominantly flexural involvement, the so-called inverse pattern (RDEB-inv). Gastrointestinal involvement may occur in all subtypes of DEB (Travis 1992).

1.8. Molecular basis of dystrophic EB.

Under the electron microscope, the cleavage plane in DEB is always found just below the lamina densa (Figures 1 and 2) and anchoring fibrils may appear reduced in number, rudimentary or absent (Tidman 1985). Hemidesmosomes are normal.

All variants of dystrophic EB appear to be due to mutations of the gene on chromosome 3 which encodes type Vll collagen (COL7A1); this is the major component of anchoring fibrils. The RDEB-HS phenotype is seen in the presence of homozygous or compound heterozygous premature termination codon mutations, which usually cause no clinical abnormality in the heterozygous state (Pulkkinen and Uitto 1999). Most patients with dominantly inherited disease have been found to have mutations causing either a glycine substitution or (less frequently) in-frame deletions of 20 - 30 amino acids of the type Vll

collagen molecule (Christiano 1994, Mellerio 1999). Not all dominantly inherited glycine substitutions result in clinical abnormalities, some causing symptoms only in the presence of an additional mutation which itself may be clinically silent when present alone (Winberg 1997, Hammami-Hauasli 1998). The nature, position and the precise combination of mutations in patients who are compound heterozygotes, or who have DDEB, has a profound effect on the severity of the phenotype (Shimizu and Masunaga1999, Murata 2000, Gardella 2002).

Other variants of dystrophic EB.

1.9. EB pruriginosa and pre-tibial EB.

These terms are used to describe DEB accompanied by itching and prurigo-like or lichenified lesions associated with scarring (Garcia-Perez 1975, McGrath 1994). The changes are most obvious on the limbs, and clinically resemble hypertrophic lichen planus. EB pruriginosa occurs in both DDEB and RDEB and a variety of causative mutations of COL7A1 have been described. Mutations do not appear to be specific for this clinically distinctive subset of DEB, as family members with the same mutation and classical features of DEB often lack pruginous lesions and do not complain of itch.

1.10. Bart's syndrome

Many individuals in a large kindred reported by Bart (1966) had localised congenital absence of skin; there was also dominant inheritance of blistering and nail dystrophy. For some years this was considered to be a separate subtype type of EB, but recent studies of members of this family have shown them to have DDEB caused by a glycine

substitution mutation of COL7A1 (Christiano 1996). Localised absence of skin is not specific for DEB as it has been noted in each of the three major subtypes of EB (Kanzler 1992).

1.11. Transient bullous dermolysis of the newborn

Some infants have extensive blistering at birth which resolves or dramatically improves during the succeeding few months (Hashimoto 1985). There is usually a family history indicating dominant inheritance. Intracytoplasmic retention of type VII collagen within basilar and suprabasilar keratinocytes is seen at the time of disease activity, but following cessation of blistering, collagen VII is confined to the dermoepidermal junction, the appearance being indistinguishable from normal skin (Fine 1993). Causative mutations of collagen VII, both heterozygous and compound heterozygous, have been identified in patients with this variant of DEB (Christiano and Fine,1997, Hammami-Hauasli 1998).

1.12. Junctional EB

Herlitz (1935) reported a recessively inherited non-scarring variant of EB in which death occurred at a few months of age. Initially, this was thought to be the same disorder as RDEB and there was diagnostic confusion until Pearson (1974) demonstrated by transmission electron microscopy that the plane of cleavage in the Herlitz variant of JEB (JEB-H) was through the lamina lucida; this was in contrast to the deeper cleavage plane found in DEB. In addition to fragility of the skin and mucous membranes, neonates with

JEB-H develop absent or dystrophic nails, often with paronychia, and granulation tissue of the nail folds and nail beds. Perioral granulation tissue is a characteristic feature in those who survive beyond 6 months of age. Unlike RDEB, there is minimal involvement of the hands and feet and very few milia. If survival is prolonged until eruption of teeth occurs, pitting or hypoplasia of dental enamel is seen. Affected infants usually fail to thrive and develop profound anaemia. Death is often due to septicaemia, but blistering or strictures of the larynx or trachea can cause sudden death. Post mortem examination reveals widespread involvement of mucous membranes, including the mouth, pharynx, oesophagus, jejunum, gall bladder, colon, anus and the urinary tract. It should be noted that post-mortem changes in the gall bladder are not specific to EB (Smith and Miller 1992).

It became apparent that some patients with recessive inheritance, extensive non-scarring blistering and a cleavage plane through the lamina lucida, survive to adult life. This type of JEB was originally termed "non-lethal JEB" but it is now included under the group heading of JEB-nH (Fine 2000). Identification of such patients during infancy on the basis of clinical features is usually impossible, and examination of the morphology of hemidesmosomes does not reliably allow distinction of lethal from non-lethal forms of JEB (Tidman 1986). The GB3 monoclonal antibody proved to be a useful aid to diagnosis, as normal staining of the dermoepidermal junction was never seen in the Herlitz variant of JEB (Schofield 1990). This antibody was later found to bind with the γ2chain of laminin 5 (see below).

Another group of individuals developing blisters at the level of the lamina lucida have a normal lifespan but their blisters heal to leave "cigarette paper-like" atrophic scarring. Blisters occur from birth, affecting both skin and mucous membranes. Involvement of hair follicles results in a scarring alopecia affecting all hair bearing areas of the body. Nail dystrophy and enamel hypoplasia occur and melanocytic naevi are seen at sites of previous blisters. This variant of JEB was initially termed generalised atrophic benign epidermolysis bullosa (GABEB) (Hintner 1982) but it is now classified as a non-Herlitz variant of JEB (Fine 2000).

A small number of patients have been described who clinically appear to have DEB but who on ultrastructural examination, are shown to have a cleavage plane through the lamina lucida. Until recently this was termed cicatricial JEB (Haber 1985), but it is now grouped with other non-lethal variants of JEB under the heading JEB-nH. Like all other JEB subtypes, inheritance is recessive.

Another group of patients have blistering from birth and pyloric atresia. Ultrastructural examination of the skin usually shows a lamina lucida cleavage plane, but occasionally the split occurs through the lowermost portion of basal keratinocytes (Mellerio and Pulkkinen1998, Wasel 2000). The disease is usually fatal within the first few months of life, even after successful surgical correction of the gastrointestinal abnormality. In those few who survive, repeated blistering within the urogenital tract can eventually cause strictures which in turn may lead to hydronephrosis and renal failure (Dank 1999).

Advances in identifying molecular abnormalities in EB have led to simplification of the classification of JEB, which now recognizes 5 clinical variants: Herlitz (JEB-H), non-Herlitz (JEB-nH), JEB with pyloric atresia (JEB-PA), an inverse phenotype (JEB-I) and late onset JEB (JEB-lo) (Fine 2000).

1.13. Molecular basis of junctional EB

1.13a. Herlitz and non-Herlitz variants of junctional EB

The lamina lucida is traversed by fine anchoring filaments which consist predominantly of laminin 5 and insert into the lamina densa (Figure 7). Laminin 5 is composed of three individual polypeptide chains - α3, β3 and γ2, each encoded by a different gene - LAMA3 (on chromosome 18q), LAMB3 (on chromosome 1q32) and LAMC2 (on chromosome 1q25-q31) respectively (Nakano 2002). JEB-H is caused by severely disruptive mutations, usually premature termination codons, in both alleles of any of the genes encoding laminin 5. Over 80% of mutations identified in JEB-H have occurred in LAMB3 (Pulkkinen and Marinkovich 1999, Nakano 2002). JEB-nH is usually due to a premature termination codon on one allele combined with a less disruptive mutation on the second allele (Nakano 2002).

1.13b. Generalised atrophic benign epidermolysis bullosa

Some patients with GABEB (now included under the heading JEB-nH) have less severe mutations of genes encoding laminin 5, but most (90%) have mutations of the COL17A1 (BPAG2) gene, found on chromosome 6p11-12 (Pulkkinen and Marinkovich 1999).

This encodes collagen XVII (the 180 kd bullous pemphigoid antigen, BPAg2), a transmembranous protein extending from basal keratinocytes across the cell membrane to the lamina densa (Figure 7) (Masunaga 1997). Although most identified mutations of COL17A1 appear to cause premature termination codons, the resulting phenotype is milder than JEB-H and compatible with normal life expectancy. Mis-sense mutations in one or both alleles cause mainly acral blistering and mild atrophy of affected skin (Schumann 1997).

1.13c. Junctional EB with pyloric atresia

JEB-PA is caused by mutations of a different hemidesmosomal gene/protein system. The α6β4 integrin is expressed in skin, gastrointestinal, respiratory and urogenital tracts(Valari 1995). Like collagen XVII, it is a transmembranous protein extending from

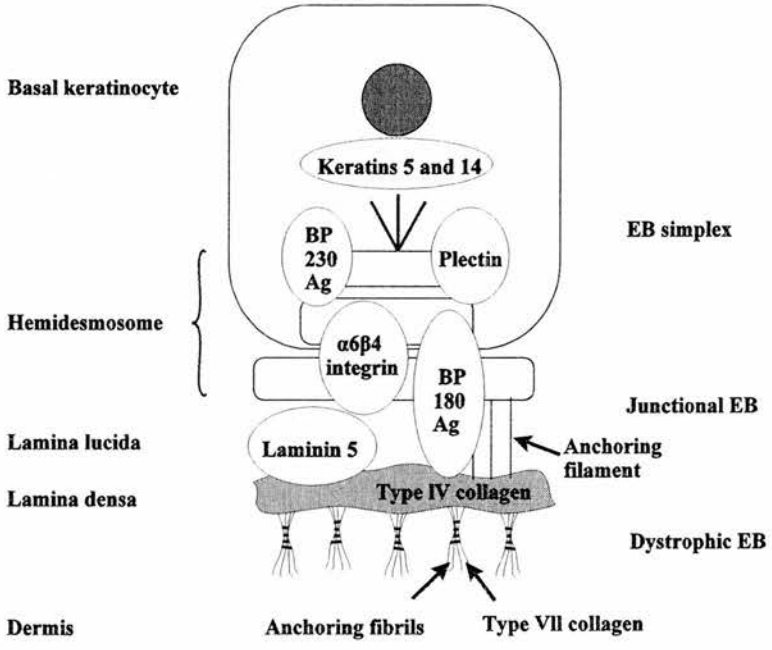


Figure 7. Localisation of the major components of the dermoepidermal junction.

the cytoplasm of basal keratinocytes to the outer plaque of hemidesmosomes (Figure 7). Intracellularly, it interacts with plectin and collagen XVII; extracellular domains of the molecule also interact with collagen XVII and additionally with laminin 5 (Jonkman 2002). Mutations of the genes (ITGA6 and ITGB4) encoding both integrin α6 and integrin β4 have been identified and, like the other variants of EB, the site and nature of the mutations appears to correlate with the severity of the phenotype. In most patients, the cleavage plane is through the lamina lucida, but a small number of patients have been reported in whom cleavage is through the lowermost portion of keratinocytes, close to the basement membrane (Mellerio and Pulkkinen1998, Wasel 2000).

Diagnosis of EB subtype by electron microscopy and a steadily increasing number of monoclonal antibodies, has led to rapid progress in understanding the structure and function of the dermoepidermal junction. Mutations of at least 10 genes are now known to cause disruption of the close interaction between keratin intermediate filaments, hemidesmosomes and collagen VII (McMillan 1998) resulting in cutaneous fragility (Table 1).

Protein	Gene	Variant of EB
Keratin 5	KRT5	EBS
Keratin 14	KRT14	EBS
Plectin	PLEC1	EBS-MD or EBS Ogna
Laminin 5 - α3 polypeptide	LAMA3	JEB-H or JEB-nH
- β3 polypeptide	LAMB3	JEB-H or JEB-nH
- γ2 polypeptide	LAMC2	JEB-H or JEB-nH
Collagen XVII	COL17A1	JEB-nH (GABEB)
α6β4 integrin - α6 polypeptide	ITGA6	JEB-PA
- β4 polypeptide	ITGB4	JEB-PA
Collagen VII	COL7A1	DEB - all subtypes

Table 1. Proteins and genes implicated in sub-types of epidermolysis bullosa.

Evidence has gradually emerged of the relative importance of the proteins encoded by these genes in maintaining the integrity of the dermoepidermal junction . Complete absence of laminin 5 or the α6β4 integrin is rapidly fatal, whilst absence of collagen XVII, collagen VII, keratin 5 or keratin 14, is compatible with survival to adult life.

1.14. Pre-natal diagnosis

The recent exciting advances have been of practical benefit to the care of patients. By determination of EB subtype it is usually now possible to accurately predict a patient's prognosis and to provide genetic counselling. If pregnancy occurs in a family known to be at risk of having an affected child, knowledge of the family-specific mutation allows pre-natal diagnosis by chorionic villous biopsy at 10 to 12 weeks of pregnancy. Termination of an affected foetus can then be offered within the first trimester. Before mutation detection was so readily available, diagnosis relied on foetal skin biopsy, a procedure still undertaken if mutations are unknown. This technique has the serious disadvantage that it must be delayed until 18-21 weeks of gestation. Before then, foetal skin is not sufficiently well developed to allow detection of diagnostic features.

Knowledge of mutation(s) in an affected family now permits use of the alternative technique of pre-implantation diagnosis (Shimizu and Suzumori 1999). In this method, which utilises in vitro fertilisation, a single cell is removed from the blastomere at the eight cell stage and mutation detection analysis is carried out. Disease-free embryos are then implanted into the uterus. Although this technique has the advantage of avoiding the need for termination of an affected foetus, it is not widely available, pregnancy rates are relatively low and it is costly.

In future, it may prove possible to search for mutations in nucleated foetal erythrocytes circulating in maternal blood, a technique which would have the great advantage of posing no risk to the pregnancy (Poon 2000, Saito 2000, Béroud 2003).

1.15. Unusual inheritance

The ability to detect mutations has led to the discovery that inheritance of EB is not always as would be predicted from the pedigree. In two families, uniparental disomy has been detected (Uitto 1999). The affected neonate in each case was homozygous for a severely disruptive mutation of LAMB3 and it was expected that each parent would be heterozygous for the same mutation. This was confirmed in the case of the mother, but the father did not carry the mutation. After non-paternity was excluded, it was concluded that the infants had each inherited two copies of the mother's single allele. The risk to any subsequent pregnancy would be no greater than in the general population. Without the benefit of mutation analyses, the risk to subsequent pregnancies in these families would have been predicted as 1:4.

Germline mosaicism in a clinically unaffected parent can also cause disease in one or more offspring and has recently been described in both JEB-H (Cserhalmi-Friedman 2002) and DDEB (Cserhalmi-Friedman 2001). Without the benefit of DNA analysis, which must include the affected child and both parents, no distinction can be made between recessive inheritance, a de-novo mutation or germline mosaicism. Each mode of inheritance is associated with very different risks to subsequent pregnancies. Recessive inheritance carries a 1 in 4 risk of a foetus being affected. In the case of a de-novo mutation, the risk to subsequent pregnancies is no higher than in the general

population, but if one parent has germline mosaicism, the likelihood of having another affected child is much higher, and depends on the ratio of mutant to wild type germ cells. If gonadal mosaicism occurs in the father, analysis of a semen sample can give an indication of the proportion of mutant germ cells, but an equivalent estimation is not possible in maternal germ cell mosaicism (Cserhalmi-Friedman 2001, 2002).

1.16. Aims of this thesis.

1. To determine the prevalence and incidence of EB in Scotland.
2. To document the spectrum of clinical features in Scottish EB sufferers.
3. To correlate the underlying genetic mutations with the clinical features.
4. To investigate the effect of EB on quality of life.

Despite the exciting advances during the last 10 years in understanding the underlying causes of EB, there is very little information about the epidemiology of these rare diseases. The most comprehensive study to date is that of the Norwegian survey of Gedde-Dahl (1970). This was an excellent survey of EB in Norway, but patients were classified at the time as having only either EB simplex (Weber-Cockayne or Köbner) or dystrophic EB. Neither junctional EB nor EBS-DM were recognised. Subsequently, after electron microscopy was carried out by Anton-Lamprecht, many families were reclassified. Although diagnostic techniques have advanced since Gedde-Dahl completed his work and the classification of EB has become more detailed, his study remains the basis for comparison by any future survey of the epidemiology of EB.

The most ambitious survey of EB is the National EB Registry, currently being compiled in the USA (Fine 1999). Although up to date diagnostic techniques, using both monoclonal antibodies and electron microscopy are available to the research team, the large size of the country and the health care system are both factors contributing to the exclusion of patients with mild disease. Because of its unique characteristics, Scotland

was selected by DEBRA for compilation of the first section of the United Kingdom EB register. This part of Britain has the advantages of being both politically and geographically well defined; its relatively stable population of 5 million (General Register Office for Scotland) is small enough to permit thorough assessment whilst being large enough to reveal the full clinical spectrum of EB.

There are numerous reports in the literature of mutations identified in a variety of EB subtypes, but there are very few studies which systematically examine mutations within a defined population. Those which do exist, whilst of interest, are small (Humphries 1996, Sørensen 1999).

During the past few years, quality of life has been assessed in a number of dermatological conditions. To those familiar with EB, it is clear that this group of disorders has a major impact on the lives of EB sufferers and their families, but to date there have been no studies of quality of life in EB.

This project, which began in 1992, sets out to investigate the epidemiology of EB and some of the less well documented clinical aspects of this group of disorders. These are subjects which have not been adequately studied, and which put into perspective the numerous and exciting laboratory discoveries in the field of EB during recent years.

Chapter Two

The Prevalence of Epidermolysis Bullosa in Scotland

2.1 Introduction

The inherited forms of epidermolysis bullosa are rare disorders and there are few published studies of their epidemiology (Table 2). The most detailed surveys have been undertaken in Norway (Gedde-Dahl 1970), Northern Ireland (McKenna 1992) and Finland (Kero 1984), countries which each have a population of fewer than 5 million inhabitants. In several other countries, assessments of the prevalence of EB have yielded low figures, suggesting incomplete sampling, differing genetic susceptibilities or pronounced regional variation. In the USA, an ambitious survey of American EB sufferers is underway and has recently reported its early results (Fine 1999). A similar register is being compiled in Italy (Tadini 1994).

Table 2. Reported prevalences of epidermolysis bullosa (per million)

	EBS	JEB	DEB
Norway [a]	24.3	-	9.3
N. Ireland [b]	28.0	0.7	3.3
Finland [c]	15.1	0.2	8.8
Croatia [d]	1.5	1.5	6.6
Japan [e]	4.0	0.2	3.5
Saudi Arabia [f]	1.7	0.0	3.7
S. Africa [g]	0.8	0.7	1.2
USA [h]	4.6	0.4	2.4

a Gedde-Dahl (1970)
b McKenna (1992)
c Kero (1984)
d Pavicic (1990)
e Inaba (1989)
f Abahussei (1993)
g Winship (1990)
h Fine (1999)

Scotland has the advantages of being politically and geographically well defined; its relatively stable population of approximately 5 million (Registrar General for Scotland, 1991) is small enough to permit thorough assessment whilst being large enough to reveal the full clinical spectrum of EB.

2.2 Methods

Scottish patients suffering from inherited forms of EB were identified from a diagnostic index at the Royal Infirmary of Edinburgh, by general practitioners and by Scottish dermatologists. All 515 Lothian general practitioners and all 38 Scottish dermatology consultants were asked to provide, with patient consent, details of any EB sufferers known to them. An advertisement directed at people suffering from blisters was placed in a newspaper distributed in rural western Scotland. Further patients were identified by probands and from routine referrals. All known affected individuals were invited to participate in the survey. Patients were interviewed and examined either in their own homes or at the Royal Infirmary of Edinburgh. Whenever possible, parents and siblings of probands were also examined. If personal contact was not feasible, patients were interviewed by telephone.

Interviews were conducted with the help of a detailed questionnaire previously devised by the Dystrophic EB Research Association (DEBRA), and answers were entered into a computerised database (Corel Quattro Pro). Classification of EB subtype was made on the basis of clinical features and where appropriate, by ultrastructural examination and antibody mapping, using the criteria suggested by Fine (1991). Patients were classified as having sporadic disease if there was no family history of EB. Details of all known EB patients living in Scotland between May 1992 and June 2001 are included in this study.

2.3 Results

Three hundred and nine patients from 103 families were identified during the study period as suffering from EB (Table 3).

Table 3. Epidermolysis bullosa sufferers identified in Scotland from 1992 - 2001.

	EBS	JEB	DEB
number of individuals	175	4	130
number of families	43	4	56

Enquiries to all 515 general practitioners in Lothian (population 726,010) at the onset of the study brought replies from 340 doctors representing 93% of practices. They were aware of only 20 of the 87 (23%) EB sufferers living in Lothian. One general practitioner notified us of a previously unidentified sufferer who would subsequently have been found by family enquiries. Of the 29 (76%) Scottish dermatologists who replied to our enquiries, 13 were aware of 37 EB sufferers. Fifteen of these patients from 12 families were not already known to us. The newspaper advertisement did not elicit any replies. The majority of patients were identified from information supplied by their affected relatives and from routine referrals to the dermatology clinic at the Royal Infirmary of Edinburgh.

For practical reasons, fifty eight patients (29 EBS sufferers and 29 DEB sufferers) could not be seen personally. Forty six of these individuals, (26 EBS and 20 DEB sufferers) were identified by probands in whom the diagnosis and subtype of EB had been confirmed. Ten had been seen at the Royal Infirmary of Edinburgh before the onset of the study (3 with EBS and 7 with DEB) and a further two patients (one with RDEB-HS

and a second with DDEB) were diagnosed at other Scottish hospitals. Twelve of the 58 unseen patients were interviewed by telephone; the remaining 251 (81%) patients were each interviewed and clinically assessed. The geographical distribution of all EB sufferers is shown in Table 4 and Figure 11.

Table 4. Prevalence of epidermolysis bullosa in Scotland in June 2001.

	EBS		JEB		DEB	
	Prevalence	(n)	Prevalence	(n)	Prevalence	(n)
Borders	215.1	(23)	0	(0)	28.0	(3)
Central (Forth Valley)	0	(0)	0	(0)	7.1	(2)
Dumfries and Galloway	0	(0)	0	(0)	20.5	(3)
Fife	17.1	(6)	0	(0)	8.5	(3)
Grampian	34.3	(18)	0	(0)	32.4	(17)
Highland	43.1	(9)	0	(0)	4.7	(1)
Lothian	42.1	(33)	1.2	(1)	67.6	(53)
Orkney	0	(0)	51.3	(1)	0	(0)
Strathclyde	27.3	(62)	0	(0)	17.2	(39)
Tayside	36.3	(14)	0	(0)	5.1	(2)
Western Isles	0	(0)	0	(0)	36.7	(1)
Unknown	-	(5)	-	(0)	-	(2)
Scotland	**33.2**	(170)	**0.3**	(2)	**24.6**	(126)

Prevalences are per million
n = numbers of individuals
(Population of Scotland = 5,114600)

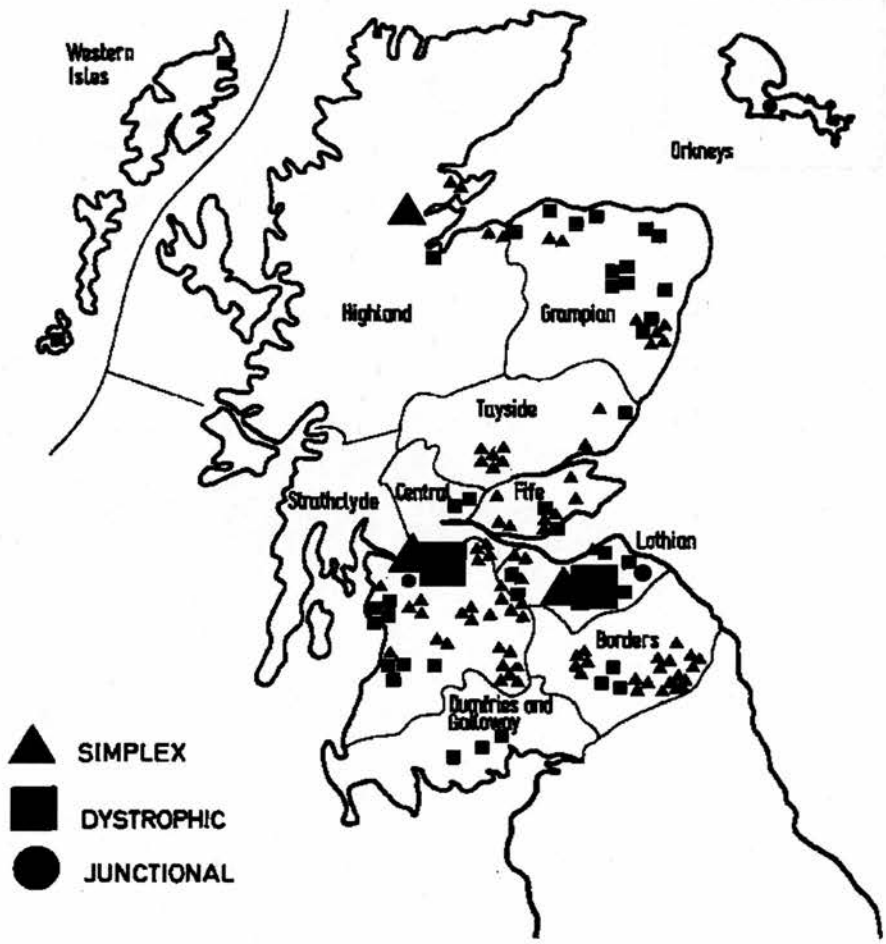


Figure 11. Location of EB sufferers within Scotland.

Large symbols represent numerous individuals; small symbols represent single individuals or small families.

2.3a Epidermolysis bullosa simplex

EB simplex (EBS) was diagnosed in 175 individuals (73 male, 102 female) from 43 families.

Eight subjects (4 male, 4 female) had the Dowling-Meara variant of EBS (EBS-DM). Ages at interview ranged from one day to 48.7 years. The eldest had an affected son, and the two youngest were identical twins, but the remaining 4 patients had no family history of EB. Examination of skin from one twin and from 4 other subjects by transmission electron microscopy showed the characteristic ultrastructural features of this condition (McGrath 1992). Ultrastructural examination of skin from the mother and son was not undertaken as a causative mutation (K14Leu122Phe) had been identified prior to her referral.

Of the remaining 167 EBS sufferers, 138 were interviewed. They were from 37 pedigrees, one with 36 and a second with 19 living affected individuals, and were aged between 1 month and 78.3 years (average 26.4 years). Forty three (30%) of the EBS patients interviewed had never consulted a dermatologist and twelve had no previous family history of the disorder. No family history is available for one child who was born in Romania and adopted by a Scottish family.

A 9 year old girl with Köbner EBS died from meningitis during the study. Two elderly adults also died and two patients with EBS-DM moved away from Scotland.

When calculating the prevalence of EBS, the 5 patients known to have died or moved away from Scotland were excluded from the figures, leaving a total EBS population of 170. Taking the population of Scotland as 5,114,600, (data from the General Register Office for Scotland), the prevalence of EBS in June 2001 is calculated as 33.2 per

million, with the EBS-DM subtype accounting for 1.2 per million. During the period 1960 - 1999, a minimum of 103 people affected by EBS were born in Scotland (this number excludes the child born in Romania). The total number of live births in Scotland during the same period was 2,995,052. Using these numbers, the incidence of EBS is calculated as 34.4 per million live births (Table 5).

Decade	Total Scottish live births	Number of affected live births	
		EBS	DEB
1990-1999	615,032	22	22
1980-1989	662,747	27	26
1970-1979	724,939	23	8
1960-1969	992,334	31	22
1950-1959	941,380	18	10
1940-1949	95,7934	11	11
1930-1939	893,357	16	9
1920-1929	1086,594	7	5
1910-1919	1139,535	4	2
1900-1909	1314,274	1	0
?	0	15	15
	9,328,126	175	130

Table 5. Live EB births in Scotland.

2.3b Junctional epidermolysis bullosa

No sufferers of JEB were known to be living in Scotland at the outset of the study, but four infants with 3 variants of this subtype of EB were subsequently diagnosed. Two, who were unrelated, had the Herlitz subtype of JEB. Both were born to healthy consanguineous parents. One, from a Pakistani family, was born in Scotland but the

second was born in Libya to Libyan parents. Skin from each child showed a cleavage plane through the lamina lucida and complete absence of staining with the GB3 antibody. Both children have since moved away from Scotland; one, now aged 7, is known to be alive, but the second has been lost to follow up. A third child, born to healthy Scottish parents not known to share a common ancestor, also had ultrastructural findings diagnostic of JEB. Staining with the GB3 antibody was present, but at reduced intensity. Although blistering remains widespread, this child is growing well and is assumed to have a non-Herlitz subtype of JEB.

A fourth child, born in England to healthy unrelated Caucasian parents, moved to Scotland shortly after successful surgical correction of pyloric atresia. He had a very mild blistering tendency. Details of this patient, who continues to live in Scotland, have been published previously (Mellerio, Pulkkinen et al 1998). Briefly, ultrastructural examination showed a cleavage plane through the lowermost portion of basal keratinocytes close to the basement membrane. Hemidesmosomes were poorly formed and sub-basal dense plates were absent . Staining with the GB-3 antibody was normal but there was reduced staining of both the α6 and β4 integrin subunits. The child was found to be a compound heterozygote for mutations of ITGB4. Both he and his mother carried a novel missense mutation in exon 3 of ITGB4 (C38R). The child was also found to have a single base-pair deletion in exon 36 (4776delG), which was present in paternal DNA and which causes a downstream premature termination codon.

The prevalence of JEB based on the two children still living in Scotland in June 2001 was 0.3 per million (Table 4). Calculation of the incidence of JEB for the period 1990-1999, based on two affected infants born in Scotland during this period, is 3.2 per million. (Table 5)

2.3c Dystrophic epidermolysis bullosa

Dystrophic EB (DEB) was identified in 130 individuals from 56 families. Four subtypes of DEB were seen. Dominant DEB (DDEB) occurred in 90 individuals (38 male, 52 female) from 19 families. The ages of the 67 interviewed patients (73%) with this subtype of DEB ranged from 2 days to 76 years (average 27 years). By the end of the study period, one patient with DEB had moved away from Scotland. Localized recessive DEB (RDEB-loc) was seen in 5 patients (2 male, 3 female) from 2 families. Four, aged between 36 and 48 years (average age 40.8 years) were interviewed. Two unrelated females, aged 47 years and 56 years suffered from the inverse variant of recessive DEB (RDEB-inv). The elder, then aged 60, died during the study from a squamous cell carcinoma of the oesophagus. The severe generalised Hallopeau-Siemens form of recessive DEB (RDEB-HS) occurred in 9 unrelated patients (3 males, 6 females). Eight, aged 3 days to 34.5 years (average age 11.4 years) were clinically assessed. Two adults in this group died during the study period; one, aged 23, died from metastatic squamous cell carcinoma and the second, aged 20, died as a result of gastric aspiration which occurred during a 'flu-like illness. Twenty thee patients (13 male, 10 female) with DEB of mild to moderate severity had unaffected parents and siblings and their inheritance pattern could not be accurately classified. Twenty patients (87%) in this group, aged from 2 months to 70 years (average 16.7 years), were interviewed. There was insufficient information to allow determination of disease inheritance in one un-interviewed child

The parents of one patient with RDEB-inv, who lived in the rural Western Isles, were shown to share a common ancestor, but consanguinity was not known in any other

families affected by DEB. One patient with mild localized DEB but unaffected parents and siblings had a cousin with severe RDEB-HS, as previously reported by Kemmett (1990). Twenty eight (41%) interviewed DDEB patients had never been seen by a dermatologist.

The prevalence of all forms of DEB in Scotland in June 2001 is calculated as 24.6 per million (DDEB17.4, DEB-unc 4.7, RDEB-HS 1.4, RDEB-loc 1.0, RDEB-inv 0.2 per million), and the incidence of DEB between 1960 and 1999, when 78 new DEB sufferers were born in Scotland, was 26.4 per million (Table 5).

2.4 Discussion

Comparison of all studies which have reported on the prevalence of EB (Tables 2 and 6) shows the highest figures for all three major variants to be in Scotland. Some caution is necessary when making such comparisons; the Norwegian survey (Gedde-Dahl 1970) was undertaken before the widespread use of electron microscopy or antibody mapping. Consequently, some patients were mis-classified. When ultrastructural examination of skin from many of the Norwegian patients was later undertaken by Anton Lamprecht (Gedde-Dahl 1990), two, originally classified as having DEB, had ultrastructural features of EBS-DM. A further 14 individuals or families thought initially to have DEB were found to have JEB, a subtype of EB which had not been recognized at the time of Gedde-Dahl's original study. In both Finland (Kero1984) and Northern Ireland (McKenna 1992), electron microscopy and antibody mapping were used. Although detection of Irish EBS patients appears to be almost as complete as in Scotland, very few Irish DEB sufferers were identified. The particularly high Scottish figure for DEB

is accounted for by the large group suffering from DDEB; it is probable that many patients with this often mild subtype of EB have gone unrecognized in other studies. Some patients with DDEB are so mildly affected that they regard their skin as normal. As the physical signs can be very subtle, careful clinical examination of all relatives of a proband is necessary if the more mildly affected family members are to be detected.

Table 6. Comparison of the epidemiology of EB in Scotland and the USA.

EB subtype	Prevalence (per million)		Incidence (per million)	
	Scotland	USA	Scotland‡	USA
All EBS	33.2	4.6	34.4	10.75
EBS WC/Kb	32.0	-	32.1	-
EBS DM	1.2	-	2.3	-
JEB	0.3	0.4	3.2*	2.0
All DEB	24.6	2.38	26.4	5.72
DDEB	17.4	0.99	16.7	2.9
All RDEB	2.6	0.92	3.0	2.04
RDEB-HS	1.4	0.42	3.0	0.41
RDEB-inv	0.2	-	-	-
RDEB-loc	1.0	-	-	-
DEB-unc	4.6	0.47	6.0	0.82

‡ based on 2,.995,052 live births in Scotland from 1960-1999
* based on 615,032 live births in Scotland from 1990-1999
(Scottish population data from the General Register Office for Scotland)

The most ambitious EB register being compiled to date is in the USA. It is not yet complete but has data on 2,214 patients (Fine 1999) (Table 6).

As in Scotland, just over 50% of their patients have EBS, but 9.2% (compared to 0.1% in Scotland) suffer from JEB and 12.8% (compared to 5.1% in Scotland) suffer from the various RDEB subtypes. Only 12.3% of their patients suffer from DDEB, whilst this

subtype accounts for 29% of our study population. These differences probably reflect the relative ease with which the most severely affected people are traced, and will become less marked as people with milder EB are included in the American register. If the prevalence of EB in the USA is similar to that in Scotland, ten thousand more American EB sufferers are yet to be traced. With the exception of Croatia (Pavicic 1990), where there is a surprisingly high prevalence of RDEB-HS, the prevalences of the recessive subtypes of EB in Scotland are broadly similar to those reported from other countries (Tables 2 and 6).

We have found a particularly high prevalence of EBS in the Borders Region (Table 4), where there are 4 apparently unrelated affected pedigrees, one having 19 affected living members. Although consanguinity might be expected in this rural area of Scotland, it has been excluded in the two families whose DNA has been studied. DNA from the remaining two families has not yet been analysed.

In other rural areas of Scotland, the prevalence of EB is consistently lower than in regions where the major cities are located (Table 4). Perhaps EB sufferers tend to gravitate towards major centres to avail themselves of medical services and opportunities for non-manual employment.

In the Lothians, there is a particularly high prevalence of EB (EBS 42.1, DEB 67.6). Part of this figure is probably accounted for by more thorough searching on our home territory where we have the benefit of an EB database and where our interest is known to local general practitioners. It seems reasonable to assume that the prevalence of EB in other urban areas of Scotland should be similar to that in Lothian, and if this is so,

many Scottish EB patients distant from Edinburgh remain undetected. Previously unknown adults suffering from EB come to light each year, confirming that these figures are undoubtedly underestimates.

The only other studies to comment on the sex ratio in EB are the British Medical Research Council study (Davison 1965) and Fine (1999) who both found a ratio of 1:1 for all EB subtypes. The slight excess of females in the Scottish study (EBS 1.4:1, DEB1.3:1) is probably due to incomplete sampling.

The Scottish study, although not the largest, appears be the most comprehensive population survey of EB yet published. It demonstrates that a large proportion of sufferers with the less severe forms of EB are unknown to both general practitioners and dermatologists, illustrating how difficult it is to achieve accurate estimates of the prevalence of EB. Increased use of computer databases in both hospitals and general practice will allow easier tracing of patients who do present to the medical profession, but many patients will remain undetected unless every relative of a proband is traced and assessed.

Chapter Three

The Clinical Spectrum of Epidermolysis Bullosa Simplex

3.1 Introduction.

Perhaps because it is often regarded as the least severe form of EB, there is relatively little documentation of the clinical features of EBS. Clinical descriptions in papers reporting newly identified mutations are often very brief and standard dermatology textbooks contain some inaccuracies. During compilation of the EB Register, it became clear that within individual pedigrees there are often marked differences in severity of disease and some variation of clinical features which cannot be accounted for by age differences. This study is an attempt to record the complete clinical spectrum of EBS within the Scottish population.

In this paper, the term "EBS-Kb" is used to describe patients who fit Köbner's (1886) original description of seasonal blistering, predominantly on the palms and soles, but occurring in addition at other sites of friction such as beneath sock garters, in the groins and under corsets."EBS-WC" is used to describe blistering confined exclusively to the palms and soles (Weber1926, Cockayne 1933). This study was completed in 1999.

3.2 Patients and Methods.

In 1999, one hundred and sixty eight individuals in Scotland were known to suffer from EBS. One hundred and forty nine were identified and assessed during compilation of the EB Register (Horn1997), and a further 19 were subsequently referred to the Department of Dermatology at the Royal Infirmary of Edinburgh (RIE). Detailed clinical information was recorded on 130 patients from 33 families. Of the remaining 38 patients, 30 were

affected relatives, identified by the probands but not yet interviewed. Information on a further 5 unrelated patients was incomplete. The remaining 3 patients were diagnosed at other Scottish hospitals as suffering from EBS, but not seen by the author. This study reports the clinical features in the group of 130 patients for whom information was complete.

3.3 Results

The three commonest sub-types of EBS were encountered in this group of Scottish patients - namely Dowling-Meara (5%), Weber Cockayne (42%) and Köbner (53%).

3.3a The Dowling-Meara subtype of EBS.

This was the most severe variant of EBS and was identified in 7 patients aged between three weeks and 49 years at initial interview. The two youngest were identical twins, but the remaining five patients were unrelated. None had an affected parent. The eldest (and only patient over 18 years of age) had an affected son, for whom no clinical information is available and who was therefore not included in the study. Blisters were congenital in four, and appeared during the first week after birth in the other three (Table 7). One of the seven was born with an area of absent epidermis on one leg. All experienced widespread blistering which, after the first few months of postnatal life, took on the clustered herpetiform appearance characteristic of EBS-DM (McGrath 1992). The severity of blistering lessened during childhood and adolescence in all patients. The four infants in the study had intraoral blistering and hoarse cries. Two have been followed up for several years; their hoarseness resolved during their third year. All subjects had

nail involvement. In the case of the infants, this consisted of periodic shedding of finger and toe nails which subsequently regrew. The adults had thickened great toe nails. None experienced any seasonal variation of the blistering tendency. Mild hyperkeratosis of the weight-bearing areas of the soles was seen in the two eldest patients, aged 17 and 49 years. Although friction from close fitting clothing was an important cause of blisters in all patients with EBS-DM, many blisters appeared to arise spontaneously. Scarring was not seen in any subjects, though widespread post inflammatory pigmentation was present in one of the two adolescents. This patient also had several atypical naevi, apparently at the sites of previous blisters.

Table 7. Age at onset of blistering in epidermolysis bullosa simplex

Age of onset	All patients	WC	Kb	DM
Birth	9	1	4	4
1-7 days	16	3	10	3
1-4 weeks	10	2	8	0
1-6 months	22	2	20	0
7 months-2 years	34	20	14	0
2-5 years	8	7	1	0
6-10 years	6	4	2	0
11-20 years	2	2	0	0
adult	0	0	0	0
unknown	23	13	10	0
Total	130	54	69	7

Ultrastructural examinations of fresh blisters from one twin and four others showed in each case characteristic cytolysis of basal and suprabasal keratinocytes and clumping of keratin tonofilaments (McGrath 1992). Identical changes were seen in a biopsy taken from the vocal cord of one infant (Shemanko 2000). The seventh and oldest patient, for whom no ultrastructural information is available, has been shown to carry a mutation (K14Leu122Phe) identified previously in other EBS-DM sufferers (Yamanishi 1994). Analysis of keratin genes has been undertaken in the remaining patients and in each case a causative mutation has been identified (K5Ser181Pro, a novel mutation [Shemanko 2000]; K14Arg125Cys [Coulombe, Stephens, Umeki, Chen, Hachisuka] found in both twins and an unrelated patient, and K14Arg125His [Coulombe, Stephens, Chan], found in two patients).

3.3b The Köbner subtype of EBS.

Sixty nine individuals, whilst experiencing blisters predominantly on the palms and soles, also blistered at other sites. Seventeen families were represented. Four patients had parents who had never blistered. Patients were aged between 1.3 and 75.5 years at interview (average 29.2 years, median 29.45 years). All experienced blisters of the feet and other sites. Sixty three (91%) suffered blisters on the palms. Intraoral blisters occurred in 17 subjects (24%) and the great toe nails were thickened in 10 patients (14%) aged between 1.5 and 57.1 years. Mild hyperkeratosis of the weight bearing areas of the soles was present in 8 adults (12%), 4 of whom were members of one family. Sixty subjects (87%) indicated that their blistering tendency was worst in warm weather. Only eleven (16%) were completely free of blisters during the winter. Three patients

(4%), two of whom were mildly affected, denied seasonal variation in severity of blistering. Six children (9%) were too young to allow assessment of seasonal variation.

Friction from clothing and jewellery was identified by patients as an important cause of blisters, with heat a contributory factor, sometimes apparently causing blisters even in the absence of friction. Improvement with age was noted by 20 patients (29%), only two of whom (aged 3 and 11 years) were under 20 years old. Fourteen patients (20%), aged 5 to 57 years, felt there had been no improvement of blistering as they had grown older. Of these, seven were over 20 years old. Eight children were too young to allow any assessment of improvement and no information is available for the remaining 27 patients (39%) whose ages range from 4 to 67 years (average 26.8 years). Whilst the age of onset of blistering in the Köbner subtype of EBS varied between birth and 10 years of age, 81% developed their first blisters before the age of 2 years (Table 7).

Two patients, mother and daughter, were each born with a single absent area of epidermis on one leg, which subsequently healed leaving subtle atrophic scarring. In one family, all 3 affected children experienced blisters in the ano-genital region. There were 6 families, none exceeding 4 sufferers, in which all affected individuals had the EBS-Kb pattern of blistering. In each of the remaining 11 pedigrees, ranging in size from 2 to 25 affected individuals, there was a mixed pattern of blistering, with both EBS-WC and EBS-Kb phenotypes represented.

3.3c The Weber-Cockayne subtype of EBS.

EBS-WC was seen in 54 patients from 20 families. Patients were aged between 1.3 and 78.3 years (average 27.8 years, median 24.6 years) at interview. Six patients had unaffected parents. Blisters were confined to the hands and feet, although 4 individuals (7%) from 3 families also experienced intra-oral blistering. Only two sufferers did not have sole involvement; one, (aged 15.5 years) had mild involvement of the palms in early childhood and the other, aged 24 years, experienced palmar blisters which were exacerbated by his manual occupation. Palmar blistering was a feature in only 31 patients (57%). Thickened great toe nails were present in seven individuals (12%), three of whom were aged under four years. No fungus was found in any nail clippings submitted for mycological examination. Five patients (9%), all adults, had mild hyperkeratosis of the soles. Seasonal variation of blistering, worst during the summer, was noticed by all but 4 very mildly affected adults (93%). Twenty seven individuals (50%) continued to experience blisters to a lesser extent during the winter, whilst 11 (20%) were completely free of blisters during the colder months. Three children were too young to allow assessment of seasonal variation and information is incomplete for the remaining 13 (24%) patients.

As in the Köbner variant of EBS, blisters were attributed to friction and heat. In nineteen patients (35%), the blistering tendency had improved with age, all but one being over 20 years old. Seventeen (31%) had not improved, though only 3 were over the age of 20. One child (aged 1.3 years) was too young to allow assessment of improvement and no information was available for the remaining 17 patients (31%),

aged 3.5 to 51 years. The age of onset of blistering was slightly later than that seen in Köbner EBS, varying between birth and the teenage years (Table 7). Fifty three percent had experienced their first blisters by the age of two years. One child was born with a localized area of absent epidermis on one leg.

There were 9 pedigrees, none exceeding 4 patients, in which all affected individuals demonstrated exclusively the EBS-WC phenotype.

3.3d Effect of EBS on lifestyle

In both EBS-WC and EBS-Kb, foot blisters frequently resulted in pain sufficient to require transport even over short distances, and to limit walking during the summer months. At home, it was common for affected children and teenagers to avoid walking; they preferred to crawl or "bottom shuffle". One adult EBS-Kb patient used a wheelchair during the summer months. Some parents carried their affected children to primary school. Teenagers often found coping with frequent changes of classroom during their school day difficult, the less motivated regularly missing school because of pain due to blisters. Two families had persuaded their local education authorities to provide taxis to take their affected children to school. Fourteen sufferers (8%) (nine with EBS-Kb, 4 with EBS-WC and one with EBS-DM), were receiving social security benefits which in eleven cases was a mobility allowance. Seven of 67 adults of employable age (10%), were unemployed, 5 of whom suffered from EBS-Kb and 2 from EBS-WC. Both adults affected by EBS-DM were in full time employment.

3.4 Discussion

Comparison of Scottish data with reported EB databases (chapter2) demonstrates that this population based study is equally if not more rigorous than other national surveys (Table 8).

Table 8. Reported prevalences of epidermolysis bullosa simplex (per million)

Country	All EBS subtypes
Scotland*	33.2
Norway [a]	24.3
N. Ireland [b]	28.0
Finland [c]	15.1
Croatia [d]	1.5
Japan [e]	4.0
Saudi Arabia [f]	1.7
S. Africa [g]	0.8
USA [h]	4.6

* figure derived from Chapter Two

a Gedde-Dahl (1970)
b McKenna (1992)
c Kero (1984)
d Pavicic (1990)
e Inaba (1989)
f Abahussei (1993)
g Winship (1990)
h Fine (1999)

We have tried to minimise any bias towards more severely affected patients by actively seeking out EBS sufferers, approximately one third of whom proved to be previously unknown to dermatologists (Horn 1997).

Localised areas of congenital aplasia cutis, originally termed "Bart's syndrome"

(Bart1966) and since recorded in all major EB subtypes (Amichai 1995) were seen in all three variants of EBS. Early onset and intraoral blisters, features typically associated with recessive dystrophic and junctional EB (Fine 1991), all occur in each variant of EBS. Widespread blistering during infancy in those few affected by EBS-DM necessitates intensive nursing care, but over the longer term, pain has a greater and more prolonged effect on the lifestyle of those affected by EBS-Kb and EBS-WC.

EBS is a spectrum, being a potentially life threatening condition for those few affected by EBS-DM, a painful disability for many and little more than an inconvenience for some. There is considerable overlap within this spectrum, to such an extent that EBS-WC and EBS-Kb should perhaps no longer be considered different disorders. Both phenotypes are seen in family members carrying identical causative mutations and this is apparent in all Scottish families of 5 or more EBS sufferers. Thus both EBS-Kb and EBS-WC appear to represent variations of the same condition. Similar as yet unexplained phenotypic variation is seen in other single gene disorders e.g. cystic fibrosis, neurofibromatosis and ichthyosis bullosa of Siemens (Stern 1995, Huson 1999, Basarab 1999). It appears the causative mutation, though important, is not the only factor determining clinical severity.

Chapter Four

The clinical spectrum of dystrophic epidermolysis bullosa

4.1 Introduction

The clinical features of RDEB-HS have long been known and are well described in standard dermatology textbooks (Rook 1999). Descriptions of the milder subtypes of DEB are less detailed, perhaps because sufferers do not come to the attention of the medical profession. As a result, there may be a tendency amongst those less familiar with EB to regard all subtypes of DEB as severe. During compilation of the EB register, many patients were encountered who had mild disease; indeed, some regarded their skin as normal. This study is an attempt to document the clinical features of DEB in all patients living within Scotland. It was completed in April 2001.

4.2 Patients and methods

Since 1992, 128 Scottish individuals have been identified as suffering from DEB. Methods of identification and assessment of patients have been described previously (Chapter 2). Diagnosis was made on the basis of clinical features, family history and in cases of doubt by antibody mapping and ultrastructural examination using the recently published revised classification system of Fine et al (2000). DDEB was diagnosed when typical clinical features were present in two or more individuals spanning at least two generations. All patients with severe clinical features and many with milder disease, underwent skin biopsy and ultrastructural assessment. Nine patients with very mild

disease and lacking a family history did not have skin biopsies and were classified as having DEB of uncertain inheritance (DEB-unc). Within the group of 128 patients, four clinical variants of DEB were identified. DDEB occurred in 88 patients (68%). Twenty four patients (19%) had no family history of DEB and clinical features of mild to moderate severity (DEB-unc). On the basis of individual pedigrees, recessive inheritance was judged to have occurred in 16 patients (13%), 9 (7%) having RDEB-HS and 7 non-Hallopeau-Siemens RDEB (RDEB-nHS). DNA from selected patients was analysed by Professor J. McGrath for the presence of COL7A1 mutations. We continue to record newly diagnosed EB sufferers, both those referred to our department and those diagnosed at other Scottish hospitals.

Ninety seven DEB sufferers from 50 families have been examined on at least one occasion and their clinical findings at initial presentation systematically recorded. Of the remaining 31 patients, 20, who have not been clinically assessed, were identified by affected relatives in whom the diagnosis of DEB had been confirmed. Nine were diagnosed at the Royal Infirmary of Edinburgh (RIE) as suffering from DEB but clinical information is incomplete. No clinical information is available for two patients diagnosed at other Scottish hospitals - one an adult suffering from DDEB and the second a neonate suffering from RDEB-HS. This study reports the clinical findings of 97 patients at their initial assessment.

4.3 Results

4.3a Dominant dystrophic epidermolysis bullosa

Detailed clinical information is available for 64 individuals from 18 families. Subjects were aged between 2 days and 76 years at interview (average age 27 years). Thirty six were female. Age at onset of blistering was known in 43 of the 64 patients and varied between birth and five years (Table 9).

Table 9. Age at onset of blistering in dystrophic epidermolysis bullosa.

Age at first blister	RDEB-HS n=8	RDEB-loc n=4	RDEB-inv n=2	DDEB n=64	DEB-unc n=19	Total
birth	7	3	1	6	4	21
1-7 days	1	0	0	12	5	18
1-4 weeks	0	1	1	0	2	4
1-6 months	0	0	0	11	6	17
7-24 months	0	0	0	10	0	10
2-5 years	0	0	0	4	0	4
unknown	0	0	0	21	2	23

Clinical features are summarised in Table 10 . With the exception of 7 children, four of whom were under 1 year old, scars were present over boney prominences in all patients. Milia were noted in 17 subjects (27%), both adults and children. Nail dystrophy, present in 44 patients (69%), was not seen below the age of 3 years. Of the 22 individuals (34%) with significant dental abnormalities i.e. excessive dental caries or its sequelae, 3 were under twenty years old and 13 reported oral blistering. A further 9 patients with oral blisters had normal dentition. Only two of the 12 (19%) subjects with anal fissures were children, the youngest being 2 years old.

Nine adults and one child aged nine years (16%) complained of dysphagia. All but one also had anal fissures (7 patients) and/or constipation (5 patients). A further six patients complained of troublesome constipation and 2 had some restriction of mouth opening. A recent barium examination in one adult with longstanding dysphagia and who intermittently produces oesophageal casts, did not reveal any abnormality. Although pseudosyndactyly was not observed in any individuals, flexion contractures of the fingers were seen in 4 adults (6%). No patients had eye involvement. Two adults (3%) had EB pruriginosa and albopapuloid lesions were apparent in 23 (36%) patients. Two heterozygous glycine substitution mutations of COL7A1 (one reported previously [Mellerio and Salas-Alanis et al 1998]) were detected in patients from 3 families.

Table 10. Clinical features of dominant dystrophic epidermolysis bullosa.

	Children aged 12 years or under (n=25) %	Adults (n=39) %
Albopapuloid lesions	8	49
Blisters	84	64
Scars	72	100
Milia	44	15
Contractures	0	10
Nail loss/dystrophy	44	85
Otitis externa	8	8
Constipation	12	21
Anal fissures	12	21
Dysphagia	4	23
Oral blisters	28	36
Dental disease	8	51
Microstomia	0	5

4.3b Dystrophic epidermolysis bullosa of uncertain inheritance

Clinical information is available for nineteen unrelated subjects. One man with mild DEB limited to the skin and oral mucosa, is the cousin of a patient with RDEB-HS who died before analysis of COL7A1 mutations was available (Kemmett 1990). The remaining patients in this group had no family history of DEB. Ages at interview ranged from 6 months to 39 years (average age 13.9). Nine (47%) had mild DEB whilst the remainder had more troublesome DEB of varying severity. All had scars, 13 (63%) had milia and 11 (58%) had nail loss or dystrophy. The youngest child to have abnormal nails was 21 months old. She was the only individual in this group born with a localised area of absent skin ("Barts syndrome") and the only one to suffer from recurrent corneal erosions. Pseudosyndactyly was not observed but mild flexion contractures of the fingers were seen in one adult. Nine subjects (47%) had albopapuloid lesions and two (11%) had EB pruriginosa. Both these individuals also had involvement of the external ear. Ten patients (53%) experienced oral blisters. Dental disease was present in 5 (26%), all but one of whom suffered oral blistering. One adult with normal dentition had both restriction of mouth opening and limitation of tongue protrusion (ankyloglossia). Anal fissures occurred in 5 individuals (26%), both adults and children, and were associated with constipation in 4. A further 3 subjects also complained of constipation. Oesophageal strictures were demonstrated in two of the four patients (21%) who experienced dysphagia, which in a third was associated with the regular production of oesophageal casts.

4.3c Recessive dystrophic epidermolysis bullosa of Hallopeau-Siemens subtype

Clinical information is available for 8 individuals who were unrelated and had unaffected parents. The cousin of one patient suffered from mild localised DEB of uncertain inheritance; details of this family have been reported previously (Kemmett 1990). The parents of one child are cousins but there was no evidence of consanguinity in the other families. Patients were aged between 3 days and 34 years at interview. Three were under 1 year old, two were children aged 3 and 10 years and three were adults aged 20, 23 and 34 years. Blisters were present at birth in all but one in whom they appeared during the following 24 hours. Two neonates also had congenital localised areas of absent skin. Though not a prominent feature, milia were visible in all patients and scars were seen on all but the youngest infant. Some features typical of RDEB-HS, including dysphagia, dental disease and the consequences of scarring (i.e. flexion contractures of the limbs, microstomia, ankyloglossia and mitten deformity of the hands), were age related, being present in all adults and the eldest child but not the younger child nor the infants. Mild flexion contractures of the fingers and some loss of inter-digital web spaces were present in the 3 year old child. All subjects, including the infants, had nail loss or dystrophy. Each adult had very sparse scalp hair and none had experienced puberty. Involvement of the external ear was seen in 3 patients. Five subjects, infants and adults, experienced corneal erosions and one adult had ectropion. Constipation proved troublesome in 6 patients, being associated in 3 with anal fissures. Only one subject, an infant, did not have oral blisters. Neither EB pruriginosa nor albopapuloid lesions were observed. Barium studies in two adults confirmed the

presence of oesophageal strictures. Analysis of DNA from one patient has identified compound heterozygous mutations of COL7A1. Since the onset of this study, the effects of scarring are becoming more apparent in the infants and dysphagia has become troublesome to such an extent that two have now received gastrostomies. Two adults have died during the study period - one from metastatic squamous cell carcinoma (SCC) arising in the skin and the second from aspiration of gastric contents during an influenza-like illness. The eldest adult has recently developed her first cutaneous SCC.

Non-Hallopeau-Siemens recessive dystrophic epidermolysis bullosa subtypes

4.3d Localised recessive dystrophic epidermolysis bullosa

Clinical information is available for 3 of 4 siblings and one unrelated patient in whom the clinical features were relatively mild and the pedigrees suggestive of recessive inheritance. The siblings have been previously reported (Marsden 1974). There is no evidence of consanguinity in either family. In each, blisters had been present at birth and there had been congenital absence of a localised area of skin in three. All blistered readily in response to minor trauma during childhood and the teenage years but reported marked improvement of skin fragility during adult life. When interviewed for this study, the four subjects were aged between 36 and 47 years. They had all experienced normal puberty and had unaffected children. All had atrophic scars over boney prominences and either nail loss or dystrophy. Milia, mild finger flexion contractures, albopapuloid lesions and external ear involvement were each seen in individual patients. Pseudosyndactyly and corneal erosions did not occur. Two sisters experienced pruritus,

which in the first was accompanied by pruriginous change over the pretibial areas and posterior shoulders and in the second affected the perineum. A number of contact sensitivities had been identified by the referring hospital in this latter patient. Oral blisters, dental disease and anal fissures were present in every patient and constipation was troublesome in 3 of the 4. Two had some restriction of mouth opening and protrusion of the tongue. Oesophagoscopy and barium swallows had previously revealed the presence of oesophageal webs in all four and in addition, oesophageal strictures had been seen in two (Marsden 1974). The siblings had required repeated oesophageal dilatations from the late teenage or early adult years, but after ten years, dysphagia resolved in two, then aged 26 and 36.

4.3e Inverse recessive dystrophic epidermolysis bullosa

Two unrelated females aged 48 and 57 years at interview were affected by flexural erosions in addition to blisters and scarring over boney prominences. The parents of the elder, an only child, shared a common ancestor from a remote part of Scotland. Milia, finger flexion contractures, loss of nails and oral blisters were seen in both patients. They also both had dental disease, ankyloglossia, mild microstomia and anal fissures. Constipation was troublesome in only one. Both patients experienced dysphagia as a consequence of oesophageal strictures and underwent repeated oesophageal dilatations. Both also suffered blistering of the pinnae and one had bilateral stenosis of the external auditory meatus associated with deafness. The elder of the two patients died during the study from a SCC arising in the oesophagus.

4.4 Albopapuloid lesions

In a significant minority of patients with DDEB (20 patients (31%) from 10 families), examination of the lumbar area revealed multiple slightly depressed areas resembling subtle atrophic scars, each measuring about about one centimetre in width and having a finely wrinkled surface (Figure 12). These were found in DDEB of all severities but were not universally present in adult members of any pedigree. They were also seen in DEB-unc (8 unrelated patients) and in one patient with RDEB-loc . The youngest subject in whom they were present was 6 years old.

In some cases, albopapuloid lesions were represented by small ivory-white papules (Pasini 1928) (Figure 13), which occurred on the upper trunk, arms and buttocks of four patients, one with mild DEB-unc and 3 unrelated individuals suffering from DDEB of a wide range of severity. Two children, one with DDEB and a second with DEB-unc, later went on to develop Pasini albopapuloid lesions during their mid teens. The youngest affected was 14 years old. Two patients complained of associated itching. Both variants of albopapuloid lesions were visible simultaneously on the skin of two individuals.

Figure 12. Atrophic albopapuloid lesions on the lumbar area.

Figure 13. Classical Pasini albopapuloid lesions on the trunk.

Figure 14. Hypertrophic linear plaques on pretibial skin in EB pruriginosa.

4.5 EB pruriginosa

Intense itching associated with the formation of pre-tibial lichenified nodules or plaques, is a distinctive clinical picture which is genetically diverse (McGrath 1994, Mellerio and Ashton1999). In our study it was seen in DEB of dominant, recessive and uncertain inheritance. The appearance may be confused clinically with both hypertrophic lichen planus and nodular prurigo. Amongst the Scottish families, itchy pre-tibial lesions were present in two unrelated adults with DDEB, and two with DEB-unc. In each of these latter patients, the lesions were remarkably extensive and linear (Figure 14). Onset of hypertrophic scarring was delayed until adult life. Perineal itching in DEB has been reported previously (Naeyaert 1995), and was seen in one Scottish adult female with RDEB-loc. Her affected sister also experiences itching but the clinical appearance in her case is that of nodular prurigo.

4.6 Discussion.

By actively seeking EB sufferers within a population we have found the prevalence of DEB in Scotland (now 24 per million) to far exceed any previous reports. The high Scottish prevalence of DDEB, although undoubtedly an underestimate, is particularly striking when compared to data from the USA (Fine 1999) (Table 11) where it is likely that the large population and different health care system may hinder full detection of EB sufferers.

Table 11. Prevalences of dystrophic EB subtypes per million in the USA (unadjusted figures) and Scotland.

	DDEB (n)	DEB-unc (n)	All RDEB (n)	RDEB-HS (n)	RDEB-nHS (n)
Scotland	17.4 (89)	4.7 (24)	2.54 (13)	1.4 (7)	1.2 (6)
USA	0.87 (216)	0.37 (91)	0.8 (98)	0.36 (90)	0.43 (108)

Despite these apparent differences, our clinical findings are broadly in agreement with those of the USA National EB Registry. Milia, a nonspecific feature of most sub-epidermal blistering disorders, were less common in Scottish DDEB sufferers, perhaps reflecting inclusion in the UK EB Register of patients with mild disease who were not actively blistering at the time of clinical examination. Otitis externa was found more frequently in all subtypes of DEB in Scotland, but the difference was particularly marked in those with RDEB-HS (37% in Scotland, 9% in the USA)and RDEB-loc (25% in Scotland and 4.1% in the USA). Other clinical features(corneal erosions, nail involvement, scarring, contractures, pseudosyndactyly, constipation, dysphagia, oral blistering, ankyloglossia and microstomia) occurred with similar frequencies to those reported from the USA (Fine 1999, Fine 2000).

With the exception of one individual, all Scottish RDEB-HS sufferers had blisters at birth, the remaining patient developing blisters within the following 24 hours. However, early blistering does not inevitably indicate either recessive inheritance or a poor prognosis. Eighteen of the 43 DDEB patients in whom the age at onset of blistering is

known, started to blister within the first week postpartum (Table 9). Although 8 (44%) of this early onset group experienced a variety of troublesome extra cutaneous features, the remaining 10 (56%) had mild disease confined to the skin. At least 37 (58%) Scottish DDEB sufferers had no blisters at birth, and 25 (39%) did not develop blisters until after the fourth week of life. This finding contrasts with the recently published revised classification of EB, which states that in DDEB it is usual for blisters to be present at birth (Fine 2000). This apparent discrepancy probably reflects more complete sampling of Scottish DDEB patients and inclusion of more patients with mild disease.

For most sufferers, DDEB does not impose serious difficulties, and many experience improvement of the blistering tendency during early adult life; some even consider their skin to be normal. Consequently there is considerable under-reporting leading to the misleading impression that DEB is inevitably a severe disorder. For many the cosmetic consequences of scarring are the most troublesome aspect of their disease, causing psychological difficulties and social embarrassment rather than physical disability. A significant minority of DDEB sufferers, in both Scotland and the USA (Fine 1999), do suffer a more severe phenotype, experiencing contractures, microstomia, dysphagia, and anal fissures, clinical features more often associated with RDEB. Gastrointestinal manifestations of DEB can occur even in the absence of active blistering and unless cutaneous signs of DDEB are sought in such patients, EB may not be apparent as the cause of anal disease or dysphagia. Not all our patients underwent barium studies, but

it is significant that in some with troublesome dysphagia and a history of producing oesophageal casts, no structural abnormality could be demonstrated.

Because scarring and its sequelae become more apparent with increasing age, some of the hallmarks of DEB may not be found in infants or children. The mitten deformity, a characteristic feature of RDEB-HS, was not seen in any child aged under four years old, though finger flexion contractures and some loss of digital web spaces were apparent by the age of three years. Nail loss or dystrophy, one of the commonest physical signs in DEB, was absent in those DDEB sufferers under 3 years old and occurred in only 15% of children with this sub-type aged 5 years or under (data not shown). Contractures, uncommon in DDEB, occurred solely in adults and dysphagia was present in only one child with this subtype.

Dental disease due to caries occurs in all subtypes of DEB and is preventable. The teeth are structurally normal (Kirkham 1996), but restricted access as a result of microstomia and pain from oral blisters both contribute to poor oral hygiene. In those suffering from dysphagia, a high carbohydrate diet may also contribute to dental decay. It is reassuring to note that of those with DDEB in this study, none of the 15 children under 5 years old and only three subjects under 20 years old have evidence of dental disease.

Albopapuloid lesions (figures 12 and 13) are a striking clinical finding in DEB, but they do not appear to be of diagnostic or prognostic significance. Although commonest in

DDEB, they also occur in other subtypes of DEB and have been reported in JEB (Eady 1983).

As yet there is no explanation for the development of the EB pruriginosa phenotype. Molecular studies have revealed a range of both heterozygous and compound heterozygous COL7A1 mutations in this distinctive clinical variant (Mellerio, Ashton et al 1999). Identical mutations have been found in DEB sufferers with a non-pruriginous phenotype, indicating that additional genetic or environmental factors, perhaps atopy, may be implicated (Mellerio, Ashton et al 1999).

The explanation for improvement of the blistering tendency or dysphagia with age is not known. Phenotypic variation within individual pedigrees is also difficult to explain. Analysis of COL7A1 mutations or of genes encoding structurally or functionally related proteins may provide some of the answers.

This study, like others, has demonstrated overlapping of clinical features between the subtypes of DEB and other variants of EB, illustrating that clinical features alone are not sufficient for diagnosis. Ultrastructural studies and antibody mapping are both useful diagnostic aids, but ultimately determination of COL7A1 mutations is essential if accurate genetic counselling is to be given to families, especially those at risk of having a severely affected child and those patients who have moderately severe DEB of uncertain inheritance. These latter patients usually prove to have mild RDEB or, less commonly, de-novo sporadic dominant mutations. More unusual inheritance patterns

such as uniparental disomy, have been reported (Uitto 1999) with profound implications when assessing risks to future pregnancies.

Challenges for the future (Uitto 2000) include preventing the devastating effects of scarring in the small proportion of severely affected DEB sufferers and also in those with milder disease for whom scars are a cosmetic embarrassment. Prevention and better treatment of SCCs, the major cause of death in RDEB (Fine 2000), are of great importance and frequent total skin inspections are an essential part of the management of those with severe DEB.

Chapter Five

Genotype-phenotype correlations in epidermolysis bullosa simplex

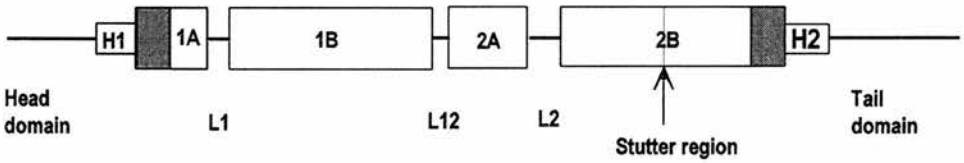
5.1 Introduction

Since the discovery that EBS is a keratin disorder (Bonifas 1991, Coulombe 1991, Lane 1992), over 50 different pathogenic mutations have been described in the genes encoding keratin 5 (KRT5) and keratin 14 (KRT14) (Tables 12 and 13). Most are missense point mutations, identified in unrelated pedigrees and resulting in dominantly inherited disease. Rarely, premature stop codons have also been reported (Gu 2002, Muller 1999). Recessively inherited EBS, due to missense or premature stop codons, has been described in a small number of consanguineous families (Hovnanian 1993, Rugg 1994, Chan 1994, Jonkman 1996, Corden 1998, Batta 2000).

Keratins are proteins which assemble to form 10 nm diameter filaments, intermediate in size between micro filaments such as actin (6nm in diameter) and microtubules such as tubulin (23 nm diameter). More than thirty different keratins have now been identified. They are expressed in the cytoplasm of epithelial cells where type I (acidic) keratins form heterodimers with compatible type II (basic) keratins. These assemble into chains which form a meshwork of filaments within the cytoplasm (Irvine 1999). Within basal keratinocytes, these filaments consist of keratins 5 and 14. After appropriate staining, they are visible on light microscopy as tonofilament bundles which weave through the cell cytoplasm. On transmission electron microscopy, tonofilaments are seen inserting into both desmosomes and hemidesmosomes. This keratin network

imparts strength and rigidity to individual keratinocytes, the epidermis and the dermoepidermal junction (Lane 1993, Uitto 1997).

Keratin 14 (K14), which is a type I acidic keratin, and keratin (K5), a type II basic keratin, are both expressed in the basal cells of stratified epithelia. Like all keratins, they share a common structure, comprising four conserved predominantly α-helical rod domains(1A,1B, 2A, 2B) connected by non-helical linker regions (L1, L12, and L2) and flanked by non-helical globular head and tail domains (Figure 15) (Lane 1993).



H1 and H2	-highly conserved regions of head and tail domains (type II keratins only)
shaded regions	-helix initiation and termination motifs
L, L12 and L2	-linker regions
stutter region	-an area of reversed polarity within the 2B α-helical rod

Figure 15. Diagrammatic keratin molecule

The amino acid sequences at the beginning and end of the central keratin rod domains (helix initiation and helix termination motifs, Figure 15), are remarkably consistent throughout the animal kingdom. In addition, helix initiation motif sequences show conservation throughout subtypes of intermediate filaments. Alterations to amino acids at these critical sites can result in abnormal filament assembly which in turn may have profound effects on cell resilience (Irvine 1999). This has been borne out by the observation that EBS-DM, the most severe subtype of EBS, is due to mutations which

alter amino acids within the helix boundary motifs of both K5 and K14. Point mutations at other sites cause either EBS-Kb or EBS-WC (Tables 12 and 13).

Table 12. Reported keratin 5 mutations in dominantly inherited EBS

EBS subtype	Keratin filament abnormalities	Mutation	Numbers of families	References
EBS-MP	Yes (mild)	K5:P25L	2	Uttam, 1996 *
EBS-MP	NR		1	Irvine, 1997 *
EBS-MP	?		2	Moog, 1999 *
EBS-MP	Yes		2	Irvine, 2001
EBS-DM	Yes	K5:V164del22	1	Rugg, 1999
EBS-WC	NR	K5:P152L	1	Muller, 1998 *
EBS-WC	Yes (mild)	K5:I161S	2	Chan, 1993
EBS-WC	NR		9	Erlich, 1995
EBS-Kb	No	K5:K173N	1	Stephens, 1995
EBS-DM	Yes	K5:L175F	1	Nomura, 1996
EBS-DM	No	K5:N176S	1	Stephens, 1997
EBS-DM	Yes		1	Sorensen, 1999
EBS-DM	Yes	K5:F179S	1	Stephens, 1997
EBS-DM	Yes	K5:S181P	1	Shemanko, 2000
EBS-Kb	Yes[d]	K5:V186L	1	Liovic,2001
EBS-WC	?	K5:N193K	1	Humphries, 1996
EBS-Kb[e]	NR	K5:V323A	1	Galligan, 1998
EBS-Kb	NR	K5:L325P	1	Sorensen, 1999
EBS-WC	Yes (mild)	K5:M327T	1	Chan, 1994
EBS-WC	?		1	Humphries, 1996
EBS-WC	NR	K5:M327K	1	Muller, 1998
EBS-WC	NR	K5:D328V	1	Matsuki, 1995
EBS-WC	NR	K5:D328H	1	Muller, 1998
EBS-WC	?	K5:D328E	1	Liovic, 2000
EBS-WC	Yes (mild)	K5:N329K	1	Chan, 1994
EBS-WC	No	K5:R331C	1	Rugg, 1993
EBS-Kb	NR	K5:L463P	1	Dong, 1993
EBS-DM		K5:I467T	1	Irvine, 1997*
EBS-Kb	Yes (mild)	K5:K472X	1	Livingstone, 2001
EBS-DM	Yes	K5:E475G	1	Lane, 1992
EBS-DM	Yes	K5:E477K	2	Stephens, 1997
EBS-DM	Yes	K5:E477X	1	Muller, 1999

Table 13. Reported keratin 14 mutations in dominantly inherited EBS

EBS subtype	Keratin filament abnormalities	Mutation	Numbers of reported families	References
EBS-WC	No	K14:K116N	3	Sorensen, 1999
EBS-DM	Yes	K14:M119T	1	Shemanko, 1998
EBS-DM[b]	No		1	Cummins, 2001
EBS-Kb	NR	K14:M119V	1	Cummins, 2001
EBS (acral only)	NR	K14:M119I	1	Chen, 1995
EBS-WC, (DM)[c]	Yes (Mild)[c]		1	Hu, 1997
EBS (generalized)	NR	K14:Q120R	1	Chen, 1995
EBS-Kb	?	K14:L122F	1	Yamanishi, 1994
EBS-DM	Yes	K14:N123S	1	Sorensen, 1999
EBS-DM	Yes	K14:R125C	1	Coulombe, 1991
EBS-DM	Yes		1	Stephens, 1993
EBS (generalized)	NR		3	Chen 1995
EBS-DM	?		1	Hachisuka, 1995
EBS-DM	?		1	Umeki, 1996
EBS-DM	Yes		1	Sasaki, 1999
EBS-DM	Yes		2	Rugg, 2000
EBS-DM	Yes		1	Ning, 2001
EBS-DM	NR		2	Hut, 2000
EBS-DM	?		1	Premaratne 2002
EBS-DM	Yes	K14:R125H	1	Coulombe, 1991
EBS-DM	Yes		4	Stephens, 1993
EBS-DM	Yes		1	Shemanko, 2000
EBS-DM	NR		1	Hut, 2000
EBS (generalized)	NR	K14:R125S	1	Chen, 1995
EBS-DM	Yes	K14:Y129D	1	Chan, 1996
EBS-Kb	No	K14:R134P	1	Rugg, 2000
EBS-Kb	NR	K14:L143P	1	Sorensen, 1999
EBS-WC	No	K14:V270M	1	Rugg, 1993
EBS-Kb	NR	K14:M272R	1	Humphries, 1993
EBS-WC	NR	K14:D273G	1	Muller, 1998
EBS (mostly acral)	NR	K14:A274D	2	Chen, 1995
EBS-WC	NR	K14: 375del	1	Chen, 1993
EBS (mostly acral)	NR	K14:I377N	1	Chen, 1995
EBS-Kb	NR	K14:L384P	1	Bonifas, 1991
EBS-Kb	?		2	Hachisuka 1995
EBS (acral only)	NR	K14:R388C	1	Chen, 1995

EBS-Kb	No	K14:E411X	1	Gu 2002
EBS-Kb	?	K14:A413T	1	Chao 2002
EBS-DM	Yes	K14:Y415H	1	Rugg, 2000
EBS-Kb	NR		1	Hut. 2000
EBS-DM	NR	K14:L419Q	1	Hut. 2000
EBS-WC	NR	K14:E422K	2	Hut. 2000

NR Not reported [a] The three families share a common haplotype.

[b] Diagnosis of EBS-DM based on clinical phenotype but not supported by ultrastructural findings.

[c] This mutation shows partial dominance. Severe keratin aggregates were seen in a skin biopsy from a patient homozygous for the mutation.

[d] Abnormal filaments detected when mutant keratin is expressed in cultured keratinocytes

[e] Some family members had an EBS-WC phenotype.

* Denotes residue number has been changes from the original publication

Although there are numerous reports of pathogenic EBS mutations (Tables 12 and 13), most document clinical findings in only single individuals from a small number of pedigrees. A few authors have looked in detail at multiple individuals within kindreds to determine if they exhibit identical phenotypes (Sørensen (1999), Sasaki (1999)). Some have observed that the location of a mutation is not the only factor to determine the severity of the phenotype and that the specific amino acid substituted also plays an important role (Cummins 2001, Sørensen 1999, Shemanko 1998). None attempt to comprehensively document the range of mutations occurring within a complete population. This chapter reports all the pathogenic K5 and K14 mutations identified within the Scottish population and assesses the correlation between phenotype and the site and nature of mutations.

5.2 Methods

During assessments of patients for the EB Register, blood samples were taken from adult EBS patients and at least one unaffected relative for extraction of DNA. In the case of two children, mouthwashes were obtained instead of blood. Initially, samples were taken only from individuals belonging to large pedigrees, but after the introduction of automated sequencing of DNA, blood samples were taken from at least one affected adult in every newly diagnosed EBS family. All samples were sent to the laboratories of Dr. David Baty and Professor E. Birgit Lane in Dundee, where DNA was extracted from peripheral lymphocytes or, in the case of mouthwashes, from buccal keratinocytes. The regions of KRT5 and KRT14 encoding the helix initiation and termination motifs were then screened for mutations. If no mutation was found, each entire gene was sequenced. By June 2002, DNA from EBS sufferers belonging to 27 (63%) of the 43 known Scottish EBS families had been extracted and sequenced. Affected members of these 27 families account for 146 (82%) of the 179 Scottish EBS sufferers identified since the onset of the study. DNA from one further family is currently undergoing sequencing.

5.3 Results

Mutations of keratin genes were found in 25 of the 27 families whose DNA was sequenced (Table 14). Twenty different mutations were identified. The majority (13) affected K14 and the remainder (7) altered amino acids in K5. Fifteen mutations were novel; three of these have formed the subjects of previous published reports (K5:R331C and K14:V270M- Rugg 1993, and K5:S181P - Shemanko 2000). One hundred and twenty affected individuals from the 27 families whose DNA has been sequenced have been clinically assessed. Their clinical features are summarized in Table 14.

Table 14. Keratin mutations in Scottish EB Simplex patients.

Family	Amino acid change	EBS sub-type	Number of individuals clinically assessed (total affected)	Sites of lesions (number of individuals affected)
16	K14:K116E*	WC & Kb	4 (4)	Feet, hands, other (3) oral (4), nails (1), anal fissures
31	K14:L122F	DM	1 (2)	Feet, hands, other, nails
30	K14:R125C	DM	1 (1)	Feet, hands, other, nails
35	K14:R125C[a]	DM	2 (2)	Feet, hands, other, nails
5	K14:R125H	DM	1 (1)	Feet, hands, other, oral
6	K14:R125H	DM	1 (1)	Feet, hands, other sites, nails
21	K14:Y129C*	Kb	6 (6)	Feet, hands, other (6), oral (2), nails (1)
12	K14:V133A*	WC&Kb	4 (4)	Feet, hands, other (4), nails (1).
11	K14:V133M*	WC	4 (5)	Feet, hands
17	K14:V133M*	WC & Kb	3 (4)	Feet, hands, other (1)
20	K14:V133L*	WC & Kb	5 (6)	Feet, hands, other (4), oral (1)
34	K14:R148C*	WC	1 (1)	Feet, nails
7	K14:V270M*	WC & Kb	4 (4)	Feet, hands (2), other (2), nails (3)
3	K14:V270M*	WC & Kb	25 (37)	Feet, hands(21), other(17), oral(4), nails (8)
39	K14:I377T*	WC	1 (3)	Feet, hands
8	K14:R388C	WC	4 (5)	Feet, hands (1)
29	K14:L418V*	WC & Kb	2 (4)	Feet, hands, other (2), oral (1)
28	K5: E170G*	Kb	2(2)	Feet, hands (1), other (2), oral(1), nails(1)
22	K5: S181P	DM	1 (1)	Feet, hands, other, oral, nails
9	K5: N193K	WC & Kb	18 (18)	Feet, hands (16), other (16),
15[b]	K5: N329S*	WC & Kb	7 (7)	Feet, hands (3), other (2) oral (3), nails (1)
1	K5: R331C	WC & Kb	6 (6)	Feet, hands (3), other (3), nails (1)
33	K5: R331C	WC	1 (1)	Feet, hands
26	K5: A428V*	Kb	1 (2)	Feet, hands, other, oral
19	K5: I467L*	WC & Kb	10 (14)	Feet, hands (7), other (2), nails (1)
13	not found	Kb	2 (2)	Feet, hands, other, oral, localized absence of skin at birth
32	not found	WC	3 (3)	Feet, hands (1)
		Total:	**120 (146)**	

* novel mutation [a] Monozygotic twins.

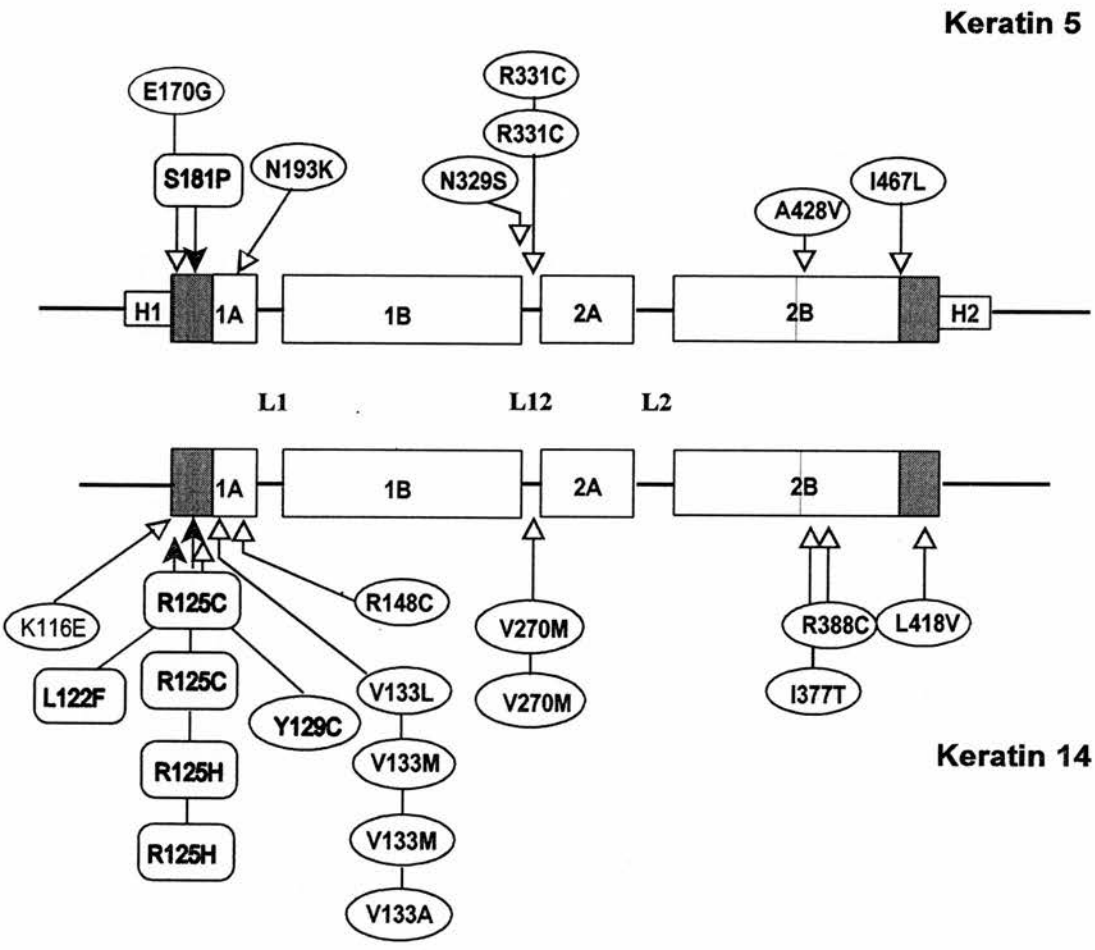
[b] Mutation only present in the two most severely affected individuals.

No mutation could be found in two families despite complete sequencing of both KRT5 and KRT14. The first of these two pedigrees comprised a mother and daughter (aged 39 and 20 years respectively) who each experienced typical seasonal blistering of the palms and soles, with minor blistering occurring at other sites of friction. Oral blisters also occurred in both. An unusual feature seen at birth in these patients was the presence of a localized area of absent skin on the shins. In each case, the defects healed rapidly, leaving subtle atrophic scarring. Studies of this family continue.

Each of 3 affected members of the second pedigree in which a keratin mutation could not be found, had typical seasonal blistering of the soles. The father (aged 36 years), but not his two children (aged 7 and 13 years) also developed occasional blisters on his hands. The nails were normal and there were no oral blisters or hyperkeratosis. These patients appeared clinically to have classical EBS.

5.3a EBS-DM

EBS-DM occurred in six pedigrees (Table 14). In each, a mutation was identified within the highly conserved helix initiation motif of either K14 or K5 (Figure 16). Four unrelated families were found to have mutations of codon 125 in K14. In two, this caused the change of arginine to cysteine (families 30 and 35 and in the remaining two, arginine was changed to histidine (families 5 and 6). In family 17, a novel mutation (K5:S161P) was found. Families 5 and 22 have been reported previously (Shemanko 2000).



Mutations resulting in the EBS-DM phenotype

Mutations resulting in the EBS Kb/WC phenotype

Figure 16. Diagrammatic keratin 5 and 14 molecules, showing the position and severity of keratin mutations in Scottish patients.

5.3b EBS Kb and EBS-WC

All other mutations occurred outside the helix initiation motifs. In nine pedigrees (families 16, 21, 11, 17, 20, 29, 28, 19 and 12) the mutation altered conserved amino acids close to these critical areas or within the helix termination motif. In family 21, blistering was particularly severe. The mutation in this family (K14:Y129C, novel mutation) lay on the boundary of helix 1A and caused a phenotype intermediate between EBS-DM and EBS-Kb. Like EBS-DM, blisters were widely distributed and herpetiform, particularly in the children. Features more in keeping with EBS-Kb and EBS-WC were the late onset of blistering (six weeks of age) and marked seasonal variation.

In family 16 (K14:K116E, novel mutation,) the phenotype was milder, although still relatively severe. This mutation also lies on the boundary of helix 1A. Blisters were extensive and easily provoked, with involvement of both the oral mucosa and genital region. A similar phenotype, though without genital involvement, was seen in family 28, which also carried a mutation in the boundary region of helix 1A (K5:E170G, novel mutation).

Four families had keratin 14 mutations within codon 133, which lies within the helix boundary region. In two, (families 11 and 17, K14:V133M, novel mutation), the phenotype was consistently mild and typical of EBS-WC. Oral blisters did not occur and only the youngest, a child aged 20 months, experienced any blistering at sites other than the palms and soles. Haplotype analysis of polymorphisms in exon 1 of K14 indicated that the mutation had arisen independently in each family (E. L. Rugg, personal communication). The remaining two pedigrees with mutations of this codon (families 20 and 12) were more severely affected, all but one of the adults blistering at sites in

addition to the palms and soles; some also experienced oral blisters. In the first of these families, valine was altered to leucine(K14:V133L, novel mutation) and in the second valine was altered to alanine.

Blistering was relatively severe in family 29 and clumping of tonofilaments was seen on ultrastructural examination. The mutation in this family (K14:L418V, novel mutation), lay within the helix termination motif of keratin 14. Although the mutation in family 19 was also within the highly conserved helix termination motif (K5:I467L, novel mutation), the phenotype was relatively mild; oral blisters did not occur and only the two youngest children of this family of 10 blistered at sites other than the palms and soles.

All other mutations occurred away from the helix boundary areas. The least severe phenotype resulting from a mutation in helix 1A was seen in family 34 (K14:R148C, novel mutation). The single affected individual had typical EBS-WC, blisters occurring only on the hands and feet. In family 9, the mutation was also within helix 1A (K5:N193K), but in contrast to family 34, the phenotype was relatively severe. For such a large pedigree (18 affected individuals), the phenotype was surprisingly consistent; there was no improvement with age and oral blisters did not occur.

A variety of phenotypes were seen in those pedigrees (families15, 7, 3, 1 and 33) who had mutations within the L12 linker region. Two adult members of family 15 (Figure 17) were particularly severely affected and were found to carry a novel mutation of KRT5 (K5:N329S).Ultrastructural examination of the skin of one showed clumping of keratin tonofilaments. Their 5 affected relatives had a very mild phenotype, blisters occurring on only the feet in 3, with additional involvement of the oral mucosa in 2 and

the hands in 1. Despite complete sequencing of KRT5 and KRT14, no keratin mutations were identified in the 5 mildly affected individuals.

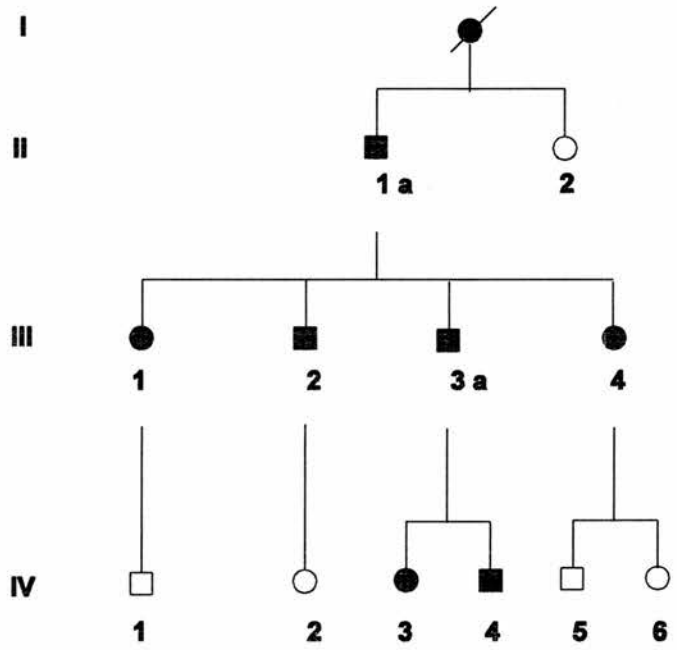


Figure 17. Pedigree of EBS Family 15
(a = severely affected individual)

The remaining four families with mutations in the L12 linker region had a mixed phenotype. Two apparently unrelated pedigrees (families 1 and 33), carried an identical novel mutation (K5:R331C). Family 1 has been described previously (Rugg 1993). Some members of this family had typical EBS-WC, whilst others, including the eldest adult, also experienced blisters at additional sites (Table 14). A mixed phenotype of similar severity was seen in the two families found to have a mutation of the K14 L12 linker region (families 7 and 3, K14:V270M, novel mutation). In family 3, which has been reported previously (Rugg 1993), four of the more severely affected patients were

over 60 years of age. Haplotype analysis indicates that the families are unrelated and that the mutations have arisen independently (Rugg, personal communication).

Mutations close to the stutter region within helix 2B were found in three pedigrees. One (K5:A428V, novel mutation) was associated with a relatively severe phenotype in the single individual available for clinical assessment (Table 14). In K14, each mutation (K14:R388C and K14:I377T, novel mutation) caused EBS-WC.

5.4 Discussion

Although not yet complete, this is the largest reported series of pathogenic KRT5 and KRT14 mutations. The Scottish population is relatively small (approximately 5 million, General Register Office for Scotland), yet a surprisingly high number (20) of different keratin mutations were found, of which 15 were novel. Addition of the Scottish mutations to the 50 previously published, increases the number of keratin mutations known to cause EBS by 23%. When all reported mutations are assessed, it is clear that whilst a few occur repeatedly in geographically widely separated areas, most are family specific. This observation has important implications when devising possible therapeutic options, including gene therapy.

Scottish mutations were clustered at known mutation "hotspots" - i.e. at the ends of the 1A and 2B rod domains, within the L12 linker region and within helix 2B close to the stutter region. Although mutations of the stutter region of K14 have been recorded previously, the mutation identified in family 26 (K5:A428V) is the first to be reported adjacent to the stutter region of K5. In keeping with published reports, alterations to amino acids within the highly conserved helix initiation motifs of K5 (codon 181) and

K14 (codons 122 and 125) caused EBS-DM, with less severe phenotypes being caused by mutations in more central areas of the rod domains. It is surprising that the mutation causing EBS-DM in family 31 (K14:L122F) has been previously reported as causing EBS-Kb (Yamanishi1994). Disruption at this highly conserved site would be expected to cause EBS-DM. However, insufficient clinical information was given in this paper describing a 24 year old patient to permit confirmation of the diagnosis of EBS-Kb and exclusion of EBS-DM.

Although the site of a mutation is critical, the nature of the amino acid substitution is also important in determining disease severity (Sørensen 1999, Cummins 2001). This is confirmed by the four Scottish families carrying mutations of codon 133 in K14. V133M was associated with predominantly acral blistering, whilst both V133A and V133L resulted in a more severe phenotype. A mutation of codon 129 in K14 (K14:Y129D) has been reported to cause severe EBS-DM (Chan 1996), but in Scotland (family 21), a different mutation of the same codon (Y129C) resulted in an intermediate phenotype showing features of both EBS-DM and EBS-K. In the literature, a range of phenotypes have been observed due to a variety of mutations within codon 119 in K14. One (K14:M119T, Shemanko 1998, Cummins 2001), caused EBS-DM, whilst a second (M119V) resulted in mild EBS-K (Cummins 2001). A third mutation in the same codon (M119I) produced only acral blistering (Chen1995).

Most members of family 19 experienced blisters on only the palms and soles, but were found to carry a mutation within the highly conserved helix termination region of keratin 5 (K5:I467L). A more severe phenotype might be expected from a mutation at this site and there is indeed a report of EBS-DM occurring as a result of a different mutation within the same codon (I467T - Irvine 1997).

Mutations within the highly conserved helix initiation motif of helix 1A do not inevitably cause EBS-DM. This is demonstrated by family 16 (K14:K116E), whose phenotype would be best classified as EBS-Kb. A different mutation in the same codon (K14:K116N) has previously been reported as causing EBS-WC (Sørensen 1999).

Two mutations at the stutter region of helix 2B in K14 each caused EBS-WC. That found in family 8 (K14:R388C) has been previously reported as causing EBS-WC (Chen 1995). The second mutation, identified in family 39 was novel (K14:I377T), but a different mutation within the same codon has also been reported as causing EBS-WC (Chen 1995).

Differences between observed and previously reported phenotypes were noted for one further Scottish pedigree (family 9). The mutation in this large family (K5:N193K) caused predominantly EBS-Kb, which appears to be in contrast to a previous report of EBS-WC due to the same mutation (Humphries 1996). Some caution in interpretation is necessary as mild EBS-Kb (i.e. blisters on the palms and soles and at other sites of friction) is sometimes labeled in the literature as EBS-WC. No clinical information was provided in the paper by Humphries et al in support of the diagnosis of EBS-WC.

Observation of large pedigrees reveals considerable intrafamilial variation in severity of phenotype. Although seldom commented on in the literature, this was noted in all Scottish families of 5 or more affected individuals. Some of the variation is accounted for by improvement with age, but many adults over 60 years old still experienced blisters at sites of friction. Perhaps the most profound intra-familial variation reported occurred in one family carrying K14:R125C. The phenotype in this family varied from EBS-DM to EBS-WC (Sasaki 1999). As such marked clinical variation can occur

between individuals with identical keratin mutations, conclusions regarding phenotype-genotype correlations should be cautious if only single individuals or small pedigrees are available for assessment.

The failure to find mutations of either KRT 5 or KRT14 in some families who clinically have classical EBS suggests that mutations of other genes, perhaps plectin (Koss-Harnes 2002) or the β4 integrin (Jonkman 2002), might cause an identical phenotype. This theory might also explain the mild phenotype seen in the less severely affected members of family 15. The mutation of the L12 linker region of KRT5 found in the two worst affected family members (K5:N329S), is not predicted to cause severe disease. Indeed, a different mutation of the same codon has caused EBS-WC (Chan 1994). The variation of disease severity in this family could be explained by all affected individuals carrying a mutation of a non-keratin gene, e.g. plectin or the β4 integrin, causing a mild phenotype; the two severely affected family members would then be compound heterozygotes, carrying both the unidentified mutation and the known KRT5 mutation. Studies of this family's DNA continue.

Analysis of DNA from Scottish EBS sufferers confirms that mutations of KRT5 and KRT14 are the cause of EBS in the majority. The failure to find keratin mutations in a small number of individuals with classical EBS indicates that mutations of one or more other genes can cause an identical phenotype in a minority. Disease severity is determined by the position of the mutation, but is also influenced by the nature of the amino acid substitution. The reasons for marked inter- and intrafamilial variation are as yet unknown.

Chapter Six

Genotype-phenotype correlations in dystrophic epidermolysis bullosa

6.1 Introduction

Dystrophic epidermolysis bullosa occurs as a result of mutations in COL7A1, the gene which encodes collagen VII. This protein is accepted as being the major, if not the only component of anchoring fibrils. It is synthesised as procollagen VII by basal keratinocytes and fibroblasts and is a homotrimer formed from three proα1(VII) monomers. Each proα1(VII) molecule consists of a central triple-helical domain flanked by non-collagenous globular N-terminal (NC-1) and C-terminal (NC-2) domains. Monomers of procollagen VII associate to form anti-parallel dimers which then align to form anchoring fibrils (Figure 18, Bruckner-Tuderman 1999).

Under the electron microscope, anchoring fibrils appear as cross-banded fan-shaped structures extending from the lamina densa (Chapter 1, Figure 18) and either looping

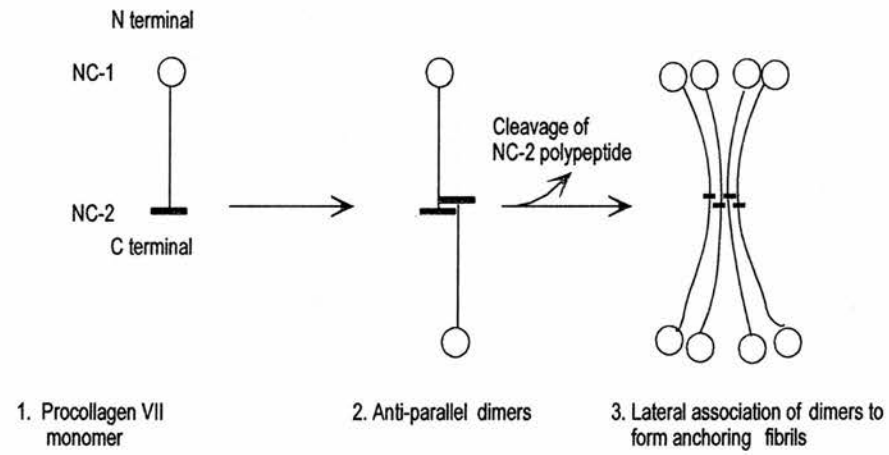


Figure 18. Diagrammatic assembly of anchoring fibrils
(adapted from Bruckner-Tuderman 1999)

down into the dermis and back to the lamina densa or embedding into anchoring plaques in the upper dermis (Bruckner-Tuderman 1999). Ultrastructural examination of skin from DEB patients shows that anchoring fibrils can appear abnormal, reduced in number or absent (Tidman 1985, McGrath 1993). To date, over 200 mutations of COL7A1 have been identified in both RDEB and DDEB (Christiano 1993, Bruckner-Tuderman 1999, Dunnill 1996, Pulkkinen and Uitto 1999, Nordal 2001, Gardella 2002). Mutations resulting in homozygous or compound heterozygous premature stop codons cause the most severe disease, either by production of truncated polypeptides which are functionally abnormal, or by decay of mRNA with consequent severely reduced levels of transcripts (Christiano and Amano 1997, Hovnanian 1997). These mutations are usually silent in the heterozygous state (Pulkkinen and Uitto 1999). Less disruptive mutations either cause mild disease or may have no clinical effect unless in the presence of a second mutation on the other allele (Shimizu and Hammamai-Hauasli).

DDEB is occasionally caused by in-frame deletions of 20-30 amino acids of the collagen VII molecule (Christiano 1994, Mellerio 1999) but most patients with DDEB have been found to carry a glycine substitution on one COL7A1 allele (Christiano 1994, Mellerio 1999). Heterozygous glycine substitutions do not inevitably cause disease; an additional mutation on the second allele may be necessary for skin fragility to occur (Winberg 1997, Hammami-Hauasli 1998, Pulkkinen and Uitto 1999, Nordal 2001). Conversely, mutations expected to cause a severe phenotype can sometimes result in surprisingly mild disease (McGrath,1999).

COL7A1 contains more exons (118) than any other gene, making detection of mutations time consuming and costly. Limited resources and a tendency to investigate mutations in only more severely affected patients have led to identification of fewer mutations in DDEB than in recessively inherited subtypes. Most dominantly inherited mutations

have been found within the triple helical collagenous domain but it appears that the site of a glycine substitution along the length of the collagen VII molecule is not related to disease severity (Pulkkinen and Uitto 1999) . With the increasing number of identified mutations, some exons, particularly exon 73, have emerged in DDEB as being common sites of pathogenic mutations (Mellerio and Salas-Alanis 1998, Mecklenbeck 1999). In recessively inherited disease, a few mutations have been detected in more than one pedigree and there appears to be some geographical clustering; R2814X, R578X and 7786delG are common in Britain (Mellerio and Dunnill 1997, Mellerio and Salas-Alanis 1998, Mohammedi 1999), 5818delC and E2857X in Japan (Tamai 1999), 2470insG in Mexico (Salas-Alanis 2000) and 497insA, 4783-1G → A , 7344G →A, 425 A→G, G1664A and 8441-14del21 in Italy (Ashton 1998, Gardella and Castiglia 2002). With these exceptions, most mutations of COL7A1 in both DDEB and RDEB appear to be family specific (Figure 19).

6.2 Methods

Patients with DEB were identified during compilation of the EB Register or in the epidermolysis bullosa clinic at the Royal Infirmary of Edinburgh. Blood samples for DNA extraction were taken from members of 15 unrelated families. In five families there was a dominant pattern of inheritance spanning at least two generations. Details of one of these families (family 35) have been published previously (Mellerio, Salas-Alanis et al, 1998). Three infants with no family history of skin disease or consanguinity, each had the severe RDEB-HS phenotype. A recessive pattern of inheritance also occurred in two adults. The first (family 5), had no affected relatives and predominantly

flexural disease; there was also oesophageal involvement. The second adult (family 8) had relatively mild but generalised disease with oesophageal involvement. His two affected siblings were not available for assessment. Two teenagers (families 7 and 41) had disease of uncertain inheritance and varying severity. The parents from family 41 and the mother from family 7 were clinically examined and found to have normal skin. Other relatives were not available for assessment. Three adults, whose relatives were not available for clinical assessment, also had disease of uncertain inheritance (families 23, 29 and 52). Two of the three, aged 33 and 72, both had the EB pruriginosa phenotype. Skin biopsies were taken from the ten patients who did not have clearly defined dominant inheritance. Biopsy specimens were submitted to transmission electron microscopy and also, in recent years, to monoclonal antibody studies, using LH7:2 (NC-1 domain of collagen VII,) GB3 (γ2 chain of Laminin 5) and COL94 (type IV collagen). Extraction of DNA and mutation analyses were kindly undertaken in the laboratories of Professor J. McGrath at the St. John's Institute Dermatology, London.

6.3 Results

6.3a Dominant dystrophic epidermolysis bullosa

All 5 families with DDEB were found to carry a glycine substitution mutation in exon 73 (Table 15). In three unrelated pedigrees, the mutations were identical (G2043R). Differing novel mutations were found in each of the two remaining pedigrees (G2026R and G2046R).

6.3b The G2043R mutation of COL7A1

There was considerable variation of phenotype both within and between the 3 pedigrees carrying this mutation. In each, individuals from three generations were affected. No scarring was visible on the skin of the youngest when she was first assessed at the age of 2 months, but by the age of three years scars were visible at the classical sites (knees, ankles, elbows and knuckles) and were seen in all remaining patients (Table 15).

Table 15. Genotype- phenotype correlations in DDEB

Family (age range in years)	Mutation	Number of individuals clinically assessed (total affected)	Clinical features - numbers of individuals affected															
			Blisters	Scars	Milia	A APL	P APL	Anal fissures	Constipation	Dysphagia	Microstomia	Oral lesions	Dental disease	Nail loss	Nail dystrophy	Contractures	Otitis externa	EB prurignosa
11 (4-65)	G2043R	10 (12)	8	10	3	5	1	0	1	0	0	3	5	1	9	0	0	1
14 (11-76)	G2043R	4 (9)	3	4	3	0	0	3	3	2	1	3	2	4	4	1	0	0
35 (0.2-50)	G2043R	3 (3)	3	2	1	2	0	2	0	2	0	2	1	2	2	0	1	0
12 (41-71)	G2026R	4 (5)	3	4	0	1	1	1	2	2	0	3	2	0	4	1	0	0
28 (7-35)	G2046V	3 (6)	2	3	1	2	0	0	0	0	1	2	2	1	3	1	1	0
Total		23 (35)																

Abbreviations:
A APL -atrophic albopapuloid lesions
P APL -Pasini albopapuloid lesions
Nail dystrophy -includes those with nail loss

All adults and children, with the exception of the two youngest, had one or more dystrophic nails, usually the great toe nail. Dysphagia occurred in 4 of 5 adult members of two of the three families (families 14 and 35). With the exception of the youngest child and eldest adult, all individuals from these two families also experienced anal

fissures. Atrophic albopapuloid lesions were seen in two of the three pedigrees (families 11 and 35), affecting 7 of 9 adults and the typical ivory-white papules described by Pasini appeared in one child as she reached adolescence. Although oral blisters occurred in adults and children from all 3 families, not every patient was affected. Finger flexion contractures and microstomia were present simultaneously in a single individual but were not seen in any other patients with this mutation. Otitis externa also occurred in one adult. One patient with the G2043R mutation showed the EB pruriginosa phenotype which clinically resembled hypertrophic lichen planus. She also experienced persistent blistering of the natal cleft.

6.3c The G2026R mutation of COL7A1

Nail dystrophy and scarring were the only features seen in every member of the single family found to carry this mutation. Skin fragility in the three eldest subjects (aged 61-71), was mild and had improved with age. One patient developed anal stenosis but none of her affected relatives experienced anal symptoms. She and her nephew (aged 41) both suffered from dysphagia which did not occur in the two remaining members of the family. Both atrophic and Pasini albopapuloid lesions were present in the nephew, whose skin blistered much more readily than that of his older relatives. He was the only member of the family to have finger flexion contractures.

6.3d The G2046V mutation of COL7A1

In two members of this family, aged 7 and 35, blistering was infrequent, causing little practical difficulty and only subtle scarring. The third member of the family (aged 28)

blistered readily and had finger flexion contractures, otitis externa and microstomia. In addition to involvement of acral sites and bony prominences, there was persistent blistering of the natal cleft. Like his 7 year old nephew, this patient also experienced oral blisters. Atrophic albopapuloid lesions were visible on the lower backs of the two adults.

6.3e Recessive dystrophic epidermolysis bullosa, Hallopeau-Siemens subtype

Novel mutations of COL7A1 were found in two infants who presented with widespread blistering at birth or within the following 24 hours. One of the two (family 43) also had a congenital localized area of absent skin. Both children went on to develop the typical RDEB-HS phenotype (Table 16).

Table 16. Scottish COL7A1 mutations in RDEB-HS

Family No.	Mutation	Exon	sub-lamina densa cleavage	LH7:2 monoclonal antibody
37	879G→T	7	confirmed	staining absent
43	8697 del 11	117	confirmed	staining absent

Biopsies of freshly blistered skin were taken from each child within 1 month of birth and revealed localization of the GB3 antigen to the roof of the blister and complete absence of staining with the LH7:2 antibody. On ultra-structural examination, the cleavage plane was confirmed to be below the lamina densa.

The mutation found in family 37 (879G →T) causes an amino acid substitution. It is a missense rather than a nonsense mutation and may be a polymorphism rather than a pathogenic mutation (personal communication, Dr. G. Ashton). No other mutations were

found in this family.

In the second child (family 43), a pathogenic mutation (8697 del 11) was found in exon 117. Deletion of 11 base pairs at this site in the gene causes disruption of the reading frame and would be predicted to result in a premature termination codon within exon118. Such a mutation is likely to lead to nonsense-mediated decay of messenger RNA and production of little or no functional protein (personal communication, Dr. G. Ashton). Although the presence of an additional mutation on the second allele is predicted in this severely affected patient, none was found.

6.3f EB pruriginosa

One patient (family 52) with no family history of skin disease presented at the age of 66 with a 20 year history of itchy hypertrophic nodules and papules over the pretibial areas and forearms. Blisters and milia were also present, but were confined to the same sites; both great toe nails were dystrophic. The skin was reported to have been normal until the age of 46 years. Light microscopy showed no evidence of lichen planus and immunofluorescence (both direct and indirect) was negative. Ultrastructural examination of the skin showed a cleavage plane through the lamina lucida. The LH7:2 and GB3 antibodies each localised to both the roof and floor of a blister, confirming a junctional split. However, analysis of COL7A1 showed a novel glycine substitution mutation G1522E which is predicted to be pathogenic (personal communication, Professor John McGrath) and which would be expected to cause sub-lamina densa cleavage, rather than the lamina lucida split observed in this patient. Because of uncertainty regarding classification of this patient's skin disorder, details of this patient have not been included in other chapters.

6.3g Patients in whom no mutation was found

Mutations of COL7A1 were unsuccessfully sought in 7 patients who clinically had a variety of subtypes of DEB (Table 17).

Table 17. DEB patients in whom no COL7A1 mutation was found

Family No.	subtype	Distinctive clinical features	sub-lamina densa cleavage	Number of individuals assessed (number affected)
5	RDEB-inv	flexural and oesophageal disease	confirmed	1 (1)
7	sporadic		confirmed	1 (1)
8	RDEB	mild disease, oesophageal involvement	confirmed	1 (3)
23	uncertain	oesophageal casts	confirmed	1 (?)
29	uncertain	EB pruriginosa	confirmed	1 (?)
41	sporadic	very mild disease	confirmed	1 (1)
45	RDEB-HS		confirmed LH7:2 at blister roof, wispy anchoring fibrils	1 (1)

6.4 Discussion

6.4a Dominant dystrophic epidermolysis bullosa

Examination of COL7A1, albeit in only a small proportion of Scottish DDEB patients, confirms exon 73 as being a common site for causative mutations of DDEB (Figure 19).

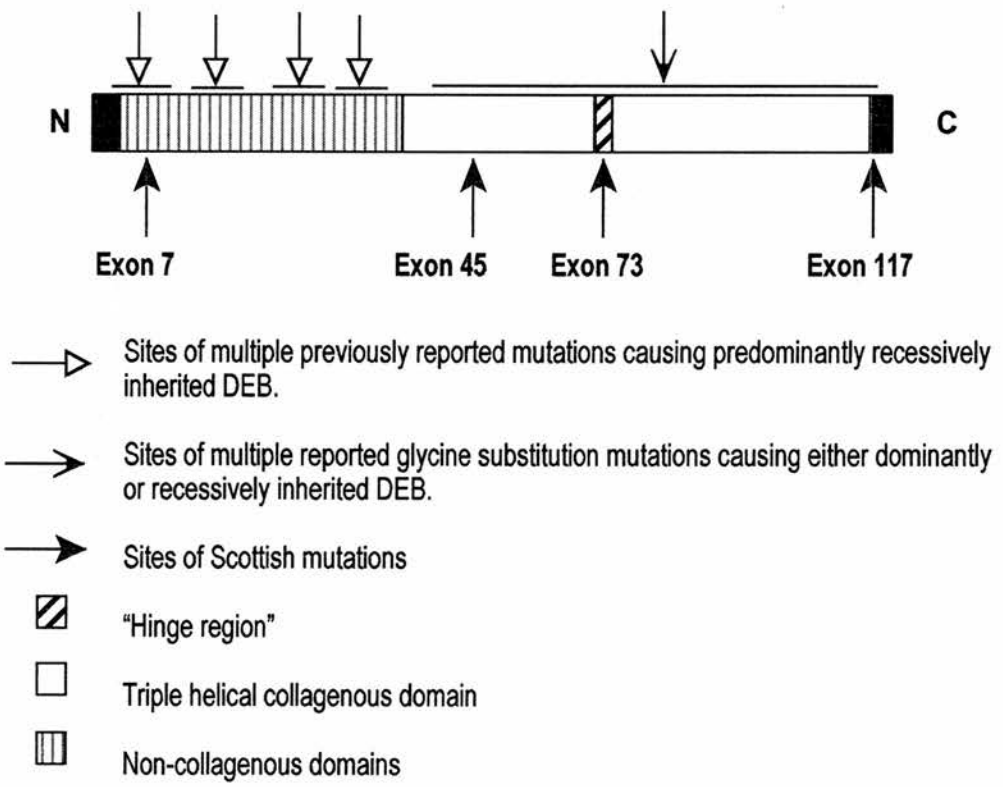


Figure 19. Diagrammatic collagen VII molecule showing positions of COL7A1 mutations in dystrophic epidermolysis bullosa

Most reported DEB mutations in the literature are family specific, but 5 point mutations (R2008, G2034W, G2043R and G2079R), all lying within exon 73, have each been identified in more than one DDEB pedigree (Christiano 1995, Cserhalmi-Friedman 1997, Hovnanian 1997, Winberg 1997, Kon 1997, Kon 1998, Rouan 1998, Mecklenbeck 1999). The most frequently reported mutation is G2043R, which has been identified in Italian, Hungarian, Norwegian, Mexican, Finnish and American families (Christiano 1995, Cserhalmi-Friedman 1997, Winberg 1997, Mellerio and Salas-Alanis 1998). This study has confirmed that the same mutation is also common in Scotland. Exon 73 lies close to a non-collagenous interruption within the triple helix, an area which is thought to act as a hinge region, providing flexibility to the collagen VII molecule (Mellerio, Salas-Alanis 1998). Replacement of glycine residues close to this critical site by bulkier amino acids appears to disrupt folding of the collagen VII molecule and its secretion by keratinocytes (Shimizu and Hammami-Hauasli 1999).

Within each of the Scottish families bearing the G2043R mutation, the severity of skin fragility is inconsistent, varying from very mild to a severity exceeding that seen in many patients with localised RDEB. A similarly broad spectrum of fragility was seen in affected members of family 28 who carry a different glycine substitution (G2046V). It is clear from this study that some unusual and distinctive clinical features of DEB,

such as Pasini albopapuloid lesions and EB pruriginosa do not occur in every person carrying a specific mutation and are often seen in only one affected family member.

The factors determining severity of DDEB in any individual remain unknown. Further analysis may reveal additional hitherto undetected mutations in the more severely affected individuals. Some might prove to be compound heterozygotes for COL7A1 mutations or, alternatively, mutations of other genes relevant to the epidermal basement membrane may additionally be present in those with more severe disease.

6.4b Recessive dystrophic epidermolysis bullosa

The single nucleotide substitution (G →T) identified in family 37 is predicted to cause an amino substitution rather than a premature termination codon. It is not typical of pathogenic COL7A1 mutations found in RDEB and is unlikely to be contributing to this patient's severe disease. The sub-lamina densa cleavage plane, absence of staining by the LH7:2 antibody together with the phenotype all point towards a severe abnormality of collagen VII but in this child, identification of causative mutations has been unsuccessful.

Like most mutations underlying both DDEB and RDEB, the COL7A1 mutation found

in family 43 appears to be family specific. It is typical of mutations identified in RDEB in that it is a small nonsense deletion which results in a premature termination codon. Because the abnormality lies in exon 117 close to the 3' end of the gene, almost full length protein should be transcribed and it is perhaps surprising that the phenotype in this patient is so severe. There are several possible explanations. Although a second mutation has not been identified, one is predicted to exist. It is known that the phenotype in RDEB is profoundly affected by the position and nature of the second mutation (Shimizu and Masunaga 1999) which in this patient may be the major disruptive influence rather than the identified mutation. It is also known that nonsense mutations lead to messenger-RNA decay and thus to low levels of polypeptide synthesis, reduced numbers of anchoring fibrils (Christiano and Amano 1997) and consequently to skin fragility. Finally, the severe phenotype in this patient could indicate that the NC-2 domain of type VII collagen is critical for correct assembly of anchoring fibrils.

It has proved frustratingly difficult to identify COL7A1 mutations in many patients who clinically and ultrastructurally have classical features of DEB, including some with severe disease. In families who lack an obvious dominant or recessive pattern of inheritance, accurate genetic counselling can be given only if pathogenic mutations have

been detected. When both parents of an EB sufferer have normal skin, disease in their child may be due to one of several modes of inheritance, each having differing implications for future pregnancies. Recessive inheritance indicates a 1 in 4 risk, in contrast to the negligible risk associated with uniparental disomy (Uitto 1999) or a de-novo mutation. In this latter situation, the mutation may later be passed on in dominant fashion by the affected child. Finally, in the case of gonadal mosaicism in one parent, the likelihood of having another affected child is much higher and depends on the ratio of mutant to wild type germ cells. If gonadal mosaicism occurs in the father, analysis of a semen sample can give an indication of the proportion of mutant germ cells, but an equivalent estimation is not possible in maternal germ cell mosaicism (Cserhalmi-Friedman 2001, 2002). When identification of COL7A1 mutations proves unsuccessful, families are denied the opportunity to make informed choices regarding future pregnancies.

Chapter Seven

Quality of life in epidermolysis bullosa.

7.1 Introduction

Skin disease can have a profound impact on many aspects of daily life. Pain may reduce mobility and affect concentration, with implications for social activities, education and employment. Visible areas of abnormal skin often arouse misunderstanding and inappropriate fear of infection amongst observers, causing embarrassment, self-consciousness and loss of confidence. Personal relationships may be affected by the demands of time-consuming treatments or by odour associated with dressings. These factors all affect quality of life (QOL), but to what degree is dependent on other considerations such as an individual's personality and coping abilities. Objective assessment of quality of life is difficult, a fact reflected by the variety of different methods that have been used in an attempt to achieve meaningful and reproducible results. During the past few years, the Dermatology Life Quality Index (DLQI) (Finlay 1994) and the Children's Dermatology Life Quality Index (CDLQI) (Lewis-Jones 1995) have emerged as useful tools for assessing the QOL of those with skin diseases, comparing severities of different disorders and judging the effectiveness of treatments (Finlay 1994, Kurwa 1995, Lewis-Jones 1995, Emerson 1998, Badia 1999, Poon 1999, Shum 2000, Touw 2001, Von der Worth 2001). The aim of this study, which was completed in June 2001, was to document the impact of different variants of EB on quality of life.

7.2 Methods

Details of all EB sufferers living in Scotland were derived from the EB Register (Horn 1997). In the first of three diagnostic groups, (group A), there were 143 EBS sufferers. One hundred and eleven adults and 27 children in this group had either EBS-WC or EBS-Kb and five patients (three adults and two children) had EBS-DM. Adults were aged between 16 and 86 years (mean 41 years) and children between 1 and 15 years (mean 10 years). In group B there were 99 DEB sufferers comprising 53 adults and 20 children with DDEB, 12 adults and 8 children with DEB-unc and 6 adults with RDEB-nHS. Adults were aged between 16 and 84 years (mean 44 years) and the ages of the children ranged from 3.5 to 15 years (mean 10 years). Six patients with RDEB-HS were considered in group C, an adult aged 40 years old and 5 children aged from 3.5 to 15 years (mean 6 years, median 3.5 years).

The DLQI and an explanatory letter stressing the anonymity of replies were posted in early May to every Scottish EB sufferer aged 16 years or over. Parents of those aged under 16 were sent a copy of the CDLQI for each of their affected children. After an interval of two weeks, a reminder was posted to every patient. To enable correlation of replies with subtype of EB, questionnaires were colour coded, different colours corresponding to each of the three diagnostic groups.

The (C)DLQI (Figures 20 and 21) contains 10 questions relating to experiences during the previous week (Finlay 1994, Lewis-Jones 1995). Each question has 5 possible answers (very much, a lot, a little, not at all or not relevant) which score 3, 2, 1, or 0 respectively. Patients were asked to state their age and sex and to tick one answer for each question; thus the maximum total score possible for any patient was 30 and the minimum was 0.

1 Over the last week, how itchy, sore, painful or stinging has your skin been?

2 Over the last week, how embarrassed or self conscious have you been because of your skin?

3 Over the last week, how much has your skin interfered with you going shopping, or looking after your home or garden?

4 Over the last week, how much has your skin influenced the clothes you wear?

5 Over the last week, how much has your skin affected any social or leisure activities?

6 Over the last week, how much has your skin made it difficult for you to do any sport?

7 Over the last week, has your skin prevented you from working or studying?

If "No", over the last week, how much has your skin been a problem at work or studying?

8 Over the last week, how much has your skin created problems with your partner or any of your close friends or relatives?

9 Over the last week, how much has your skin caused any sexual difficulties?

10 Over the last week, how much of a problem has the treatment of your skin been, for example by making your home messy or by taking up time?

Figure 20. The Dermatology Life Quality Index questionnaire.

1. Over the last week, how itchy, "scratchy", sore or painful has your skin been?

2. Over the last week, how embarrassed or self conscious, upset or sad have you been because of your skin?

3. Over the last week, how much has your skin affected your friendships?

4. Over the last week, how much have you changed or worn different or special clothes/ shoes because of your skin?

5. Over the last week, how much has your skin trouble affected going out, playing or doing hobbies?

6. Over the last week, how much have you avoided swimming or other sports because of your skin trouble?

7. Last week, was it either :
   school ☐
   or (answer only one)
   holiday time ☐

   If school time: over the last week, how much did your skin affect your school work?

   If holiday time: how much over the last week, has your skin interfered with your holiday plans?

8. Over the last week, how much trouble have you had because of your skin trouble, with other people calling you names, teasing, bullying, asking questions or avoiding you?

9. Over the last week, how much has your sleep been affected by your skin problem?

10. Over the last week, how much of a problem has the treatment of your skin been?

Figure 21. The Childrens' Dermatology Life Quality Index questionnaire

7.3 Results

Replies were received from 75 patients in group A (57 adults and 18 children), a response of 52%, (adults 50% and children 62%). In group B, there were 32 replies from adults (45%) but only 8 replies (29%) from children, an overall response of 40%. Only one of the patients in group C, a child, did not reply (response rate of 83%). Overall, the response was 48%. As many patients failed to state their age or sex, no attempt was made to correlate severity of symptoms with these parameters.

Adults

7.3a EB simplex

The mean total score in group A was 10.7 (range 0 to 26, median 10). Question 1, which enquired about symptoms, had the highest mean score (1.6, median 2) and question 6, relating to sport, also recorded high scores (mean 1.5, median 2, Figure 22).The lowest mean score (0.3), was for question 9 which asked about sexual difficulties. Four patients, aged between 23 and 39, each scored a total of zero.

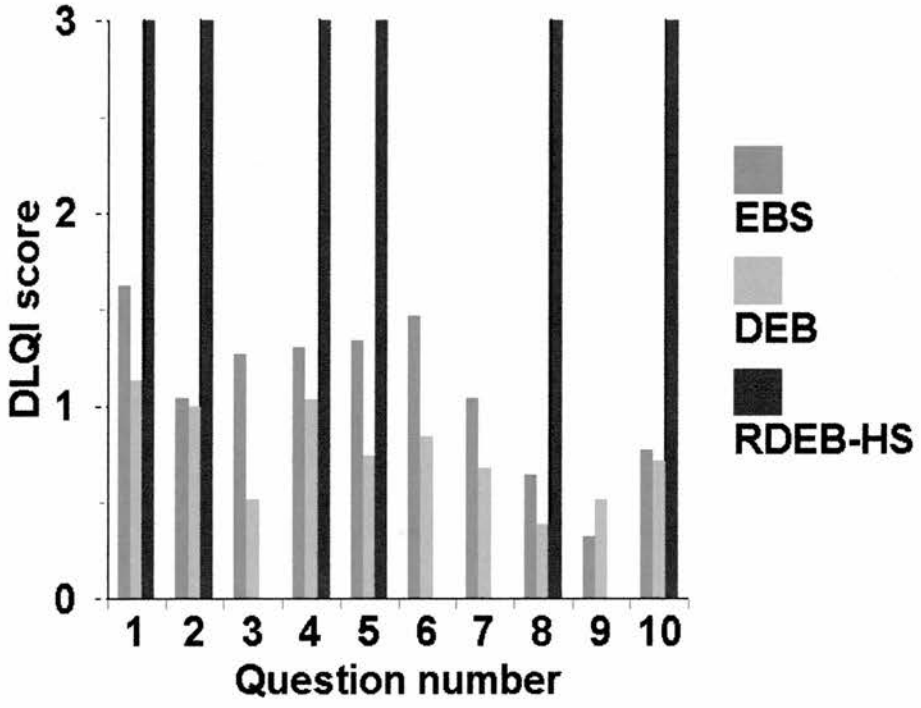


Figure 22. Mean DLQI scores in adult EB patients

The question answered "very much" most often (20 patients, 35%) was number 6 , which related to sport (Table 18).

		Question number									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
EBS (n=57)	n	12	7	7	7	9	20	13	4	1	1
	%	21	12	12	12	16	35	23	7	2	2
DEB (n=32)	n	4	4	2	3	2	7	3	1	2	2
	%	13	13	7	10	7	23	10	3	7	7
RDEB-HS (n=1)	n	1	1	0	1	1	0	0	1	0	1
	%	100	100	0	100	100	0	0	100	0	100

Table 18. Numbers of adults scoring "3" for individual questions.

7.3b Dystrophic EB

The mean total score was 7.5 (median 4, range 0 to 28). Questions 1, 2 and 4 (symptoms, embarrassment and clothing) achieved the highest mean scores of 1.2, 1.0 and 1.0 (median scores of 1 in each case) and question 8 (relationships) had the lowest mean score (0.4, median 0, Figure 20). Like EBS, the question awarded "very much" by the greatest number of patients was number 6 (sport), which was selected by 7 (23%) patients. Responses to each individual question varied from 0 to 3. Five patients, 3 aged between 67 and 84 and two of unknown age, recorded total scores of zero.

Children

7.3c EB Simplex

Children with EBS recorded a mean total score of 15 (median 15, range 0 to 30). Question 1 achieved the highest mean score (2.1, median 2), followed by question 5 (play and hobbies) (mean 1.9, median 2, Figure 21). The lowest score was for question 8,which enquired about teasing and bullying (mean 0.9, median 0.5). Only one patient (aged 8.5 years) recorded a total score of 0.

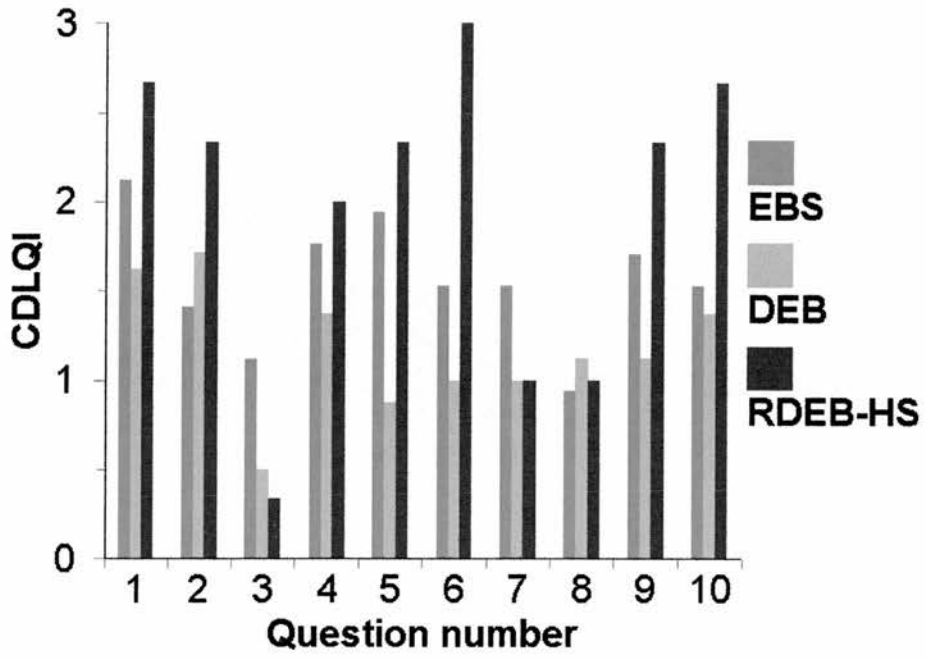


Figure 23. Mean CDLQI scores for children with EB

In keeping with the adult responses, the question awarded "very much" by the greatest number of children (9 children, 50%) was number 6 (Table 19). The full range of answers was seen in response to every question.

		Question number									
		1	2	3	4	5	6	7	8	9	10
EBS (n=18)	n	6	4	4	5	7	9	3	2	5	3
	%	33	22	22	28	39	50	17	11	28	17
DEB (n=8)	n	3	2	0	1	0	1	0	2	1	0
	%	38	25	0	13	0	13	0	25	13	0
RDEB-HS (n=4)	n	3	2	1	1	2	4	1	0	2	3
	%	75	50	25	25	50	100	25	0	50	75

Table 19. Numbers of children scoring "3" for individual questions

7.3d Dystrophic EB

The mean total CDLQI score for children with DEB was 11.5 (median 12.5, range 0 to 23).Question 2 (embarrassment and sadness) recorded the highest mean score (1.7, median 2), followed by question 1(symptoms) (mean1.6, median 2). The lowest mean score was for question 3, which asked about friendships (mean 0.5, median 0). Question number 1 was awarded the maximum score by the most patients (3 patients, 37.5%). One child, aged 8, scored a total of zero. No patients scored higher than 2 for questions 3, 5, 7 and 10, but the remaining questions were awarded the full range of scores.

7.3e RDEB-HS in adults and children

The one adult with RDEB-HS recorded a total score of 18. Question numbers 3,6,7,and 9 were each awarded a score 0, all remaining questions scoring 3.

The four children with RDEB-HS scored a mean total of 22.0 (median 21, range 17 to 29). All patients answered "very much" to question 6 (sport). Question 3 (friendships) recorded the lowest mean score (mean 1.0, median 1).

7.4 Discussion.

Despite the limitations of a postal survey and the possibility of a biassed response, the results reflect the wide variety of problems encountered by EB sufferers. Adults and children with both EBS and DEB experience an impairment of QOL equivalent to that caused by moderate to severe psoriasis and atopic eczema (Table 20). Like most other skin disorders, question 1 relating to symptoms was scored most highly by all those in groups A and B. Comparison with other studies suggests that RDEB-HS causes greater impairment of QOL than any skin disease previously assessed (Table 20).

	Adults	Children
Atopic eczema	4.14 [1] -16.2 [2]	7.7 [3] -12.7 [4]
Psoriasis	4.5 [1] -13.9 [2]	-
Hidradenitis suppurativa	8.9 [5]	-
Urticaria	7.5-15 [6]	-
Behçet's syndrome	5.7 [7]	-
RDEB-HS	18.0	22.0
EBS	10.7	15
DEB	7.5	11.5

References:

1. Badia (1999)
2. Kurwa (1995)
3. Lewis-Jones (1995)
4. Emerson (1998)
5. Von der Worth (2001)
6. Poon (1999)
7. Blackford (1997)

Table 20. Mean (C)DLQI scores in skin disorders.

Inevitably, the number of RDEB-HS patients assessed in this study was small but they were representative of those having this rare disorder. Their high QOL scores are unlikely to be overestimates and in some cases were artificially lowered by responses "not at all" or "not relevant" to questions on matters entirely incompatible with the lifestyle of RDEB-HS suffers. These include sport, sexual difficulties and domestic duties.

The devastating effects of RDEB-HS are obvious to observers, but the difficulties and distress experienced by sufferers of less severe variants of EB may not be so apparent.

EBS is often considered to be the mildest subtype of EB but this survey indicates that it has a more marked effect on QOL than the non-Hallopeau-Siemens variants of DEB. Patients' replies reflect the greater restriction of physical, social and sporting activities caused by EBS compared to DEB and also its greater impact on employment and education. Despite the often visible skin lesions of DEB, children with EBS, whose blisters are usually concealed by footwear, experience more difficulties with friendships. Pain prevents participation in the more physical aspects of normal childhood and can contribute to social isolation and psychological problems. Undoubtedly, some DEB sufferers, both those with DDEB and those with RDEB-nHS, do experience great difficulties, but such patients are few in number, the majority of DDEB patients and even some with RDEB-nHS having only mild disease (Horn 2002).

Although some patients with EBS experience an improvement of the blistering tendency during adult life (Horn 2000), many do not and for them, impairment of QOL is lifelong.

Chapter Eight

Conclusions

Tracing EB patients in Scotland has led to compilation of the most comprehensive EB Register of any yet reported. Although the American EB Registry includes details of a greater number of EB sufferers, the prevalences of EBS and DEB in Scotland are far higher, particularly amongst those with DDEB suggesting that in Scotland detection of EB patients is more complete.

Many Scottish EB sufferers (EBS 30%, DDEB 41%) were brought to the attention of dermatologists for the first time directly as a result of this study. Despite this exhaustive attempt to accurately assess the epidemiology of EB in Scotland, previously undiagnosed EB sufferers are still occasionally referred to clinics at the Royal Infirmary of Edinburgh, demonstrating that the high prevalence figures for EB in this study remain an underestimate. Nevertheless, the data has proved to be of practical use in the planning of healthcare services for EB patients not only in Scotland but also, by extrapolation, throughout the United Kingdom. In the future, the Register should allow identification of those Scottish EB patients who might benefit from novel treatments.

Systematic clinical evaluation of Scottish EBS patients has highlighted some differences from traditional clinical descriptions. Oral blisters and nail dystrophy in EBS-DM have long been recognized, but standard dermatology textbooks do not mention that these clinical features are also found in a substantial minority of patients with the less severe variants of EBS. Blisters at sites other than the palms and soles were considered to occur in only a small minority of those with non-Dowling-Meara subtypes of EBS (Rook

1999). This study has shown that at least 43% of such patients develop blisters at other sites of friction. Assessment of entire families with EBS has shown a spectrum of severity which is only partially determined by the location and nature of the underlying mutation. The occurrence of both Weber-Cockayne and Köbner phenotypes simultaneously in all but the smallest EBS pedigrees suggests that these are one disorder, whose severity is modified in an individual by other, currently unidentified, factors. The terms "EBS/WC" and "EBS/Kb" are not sufficiently precise to be used alone but should always be accompanied by a clinical description.

Similar and often marked intra familial variation of severity is also seen in DDEB. Some unusual and distinctive clinical features of DEB, such as Pasini albopapuloid lesions and EB pruriginosa do not occur in every person carrying a specific mutation and are often seen in only one affected family member. As in EBS, the factors determining severity of DDEB in an individual remain unknown. Such extensive clinical variation indicates that unless several affected individuals can be assessed, caution is necessary when attributing a phenotype to a newly described pathogenic mutation.

As a result of assessment of EBS patients in Scotland, the worldwide database of pathogenic keratin mutations has been increased by 23%. A small number of patients with classical EBS were identified in whom no keratin mutations were found, suggesting that mutations of other genes can cause identical phenotypes to those induced by mutations of KRT5 and KRT14.

Because analysis of COL7A1 is less readily available, fewer mutations were identified in DEB patients. It is frustrating that mutation detection failed or was only partially successful in approximately 50% of those DEB patients whose DNA was analysed,

including some with severe disease. Further detailed examination of COL7A1 in these patients may reveal additional mutations. If mutations of this gene cannot be found, analysis of genes encoding other basement membrane proteins, eg.α6 or β4 integrins, or collagen XVII, may show some of these patients to be compound heterozygotes for mutations of two different genes. At present, there are no routine facilities in the United Kingdom for DNA analysis of these patients, who, despite the genetic basis of their disease, are denied the benefits of informed genetic counselling.

Examination of the QOL of EB patients confirmed the profound impact of RDEB-HS on QOL. Perhaps more surprising was the finding that EBS, often considered the mildest EB subtype, also seriously reduces QOL, especially in children. DEB (excluding RDEB-HS) had less impact on quality of life, and for many, was a mild disease.

This study confirms the need to investigate the mechanisms underlying scarring in DEB. For many this is only a cosmetic problem, but in an unfortunate minority, it is sufficiently severe to cause deformity and disability. The aggressive nature of SCC in DEB is also not understood and current treatments are inadequate. It is to be hoped that current research will prove constructive in both areas. There is also an unmet need to provide a mutation screening service for those DEB patients who have severe disease or disease of uncertain inheritance.

Appendix 1

Numbers of Epidermolysis Bullosa patients in Scotland

EBS

Total seen 1992-2001	= 146
Total not seen	= 29
	175
Total died/moved	= 5
Total population June 2001	**= 170**

DEB

Total seen 1992-2001	= 101
Total not seen	= 29
	130
Total died/moved	= 4
Total population June 2001	**= 126**

JEB

Total seen 1992-2001	= 4
Total moved	= 2
Total population June 2001	**= 2**

Appendix 2

Demographic details of epidermolysis bullosa simplex patients

patient number	family no	initials	m	f	EBS sub-type	age of onset	age at interview (years)	region
1	1	JB	0	1	Kb	6-10 yrs	23.8	Lothian
2	1	CB	0	1	WC	6-10 yrs	17.8	Lothian
3	1	MB	0	1	Kb	6-10 yrs	47.5	Lothian
4	1	AB	1	0	WC	6-10 yrs	37	Lothian
5	1	DM	0	1	WC	6-10 yrs	26	Lothian
6	1	SM	1	0	Kb	7mths-2 yrs	2.7	Lothian
7	2	AM	0	1	WC	2-5 yrs	15.5	Lothian
8	3	PK	1	0	WC	7mths-2 yrs	22.2	Lothian
9	3	KM	0	1	Kb	1-4 wks	7.8	Strathclyde
10	3	KvM	1	0	Kb	1-4 wks	10.1	Strathclyde
11	3	AM	1	0	WC	7mths-2 yrs	34.3	Strathclyde
12	3	LM	1	0	Kb	1-7 days	4.3	Lothian
13	3	RD	1	0	Kb	birth	41.2	Lothian
14	3	Mg W	0	1	Kb	1-4 wks	38.8	Lothian
15	3	MW	0	1	WC	1-4 wks	16.3	Lothian
16	3	St W	1	0	Kb	1-4 wks	13.8	Lothian
17	3	Sn W	1	0	Kb	1-4 wks	13.8	Lothian
18	3	HR	0	1	Kb	?	62.3	Lothian
19	3	AK	0	1	Kb	1-7 days	57.2	Lothian
20	3	LB	0	1	WC	7mths-2 yrs	14.8	Grampian
21	3	AB	0	1	WC	?	35.5	Grampian
22	3	VB	0	1	WC	7mths-2 yrs	16.8	Grampian
23	3	GH	1	0	Kb	7mths-2 yrs	34.3	Tayside
24	3	Gr H	1	0	Kb	1-6 mths	4.3	Tayside
25	3	AH	0	1	Kb	?	61.7	Tayside
26	3	EH	0	1	Kb	1-6 mths	2.6	Tayside
27	3	RH	1	0	Kb	1-6 mths	30	Tayside
28	3	SH	1	0	Kb	?	1.9	Tayside
29*	3	ZH	0	1	?	1-4 wks	1.3	Tayside
30	3	IM	1	0	Kb	2-5 yrs	61.8	Fife
31	3	AS	1	0	Kb	1-7 days	50.6	Fife
32	3	AM	0	1	WC	?	67.6	Fife
33*	3	JM	0	1	?	1-6 mths	1.1	Fife
34	3	GM	1	0	WC	7mths-2 yrs	16.5	Fife

patient number	family no	initials	m	f	EBS sub-type	age of onset	age at interview (years)	region
35	4	KM	1	0	WC	1-7 days	23.7	Strathclyde
36	4	AM	0	1	Kb	1-6 mths	3	Strathclyde
37	5	DC	1	0	DM	birth	0.1	Lothian
38	6	CC	0	1	DM	1-7 days	11.5	Strathclyde
39	7	AJ	0	1	Kb	7mths-2 yrs	26.6	Lothian
40	7	WT	0	1	Kb	7mths-2 yrs	57.1	Lothian
41	7	HJ	0	1	WC	7mths-2 yrs	3.5	Lothian
42	7	SJ	0	1	WC	7mths-2 yrs	3.5	Lothian
43	8	MG	0	1	WC	7mths-2 yrs	51.5	Strathclyde
44	8	MC	0	1	WC	7mths-2 yrs	29.3	Strathclyde
45	8	PC	1	0	WC	7mths-2 yrs	5.3	Strathclyde
46	8	BD	1	0	WC	7mths-2 yrs	24.9	Strathclyde
47*	8	ED	0	1	WC	?	2.6	Strathclyde
48	9	DP	0	1	Kb	1-6 mths	17.8	Borders
49	9	EP	0	1	Kb	1-6 mths	35.3	Borders
50	9	NL	0	1	Kb	1-6 mths	66.8	Borders
51	9	DP	0	1	Kb	7mths-2 yrs	58.5	Borders
52	9	RR	0	1	Kb	?	65.6	Borders
53	9	BA	0	1	Kb	?	30.3	Borders
54	9	CA	0	1	Kb	1-4 wks	10.8	Borders
55	9	VP	0	1	Kb	7mths-2 yrs	7.8	Borders
56	9	BP	1	0	WC	?	38	Borders
57	9	TH	1	0	Kb	?	63.1	Borders
58	9	TH Jnr	1	0	Kb	1-7 days	34.3	Borders
59	9	CJ	0	1	Kb	?	73	Borders
60	9	AC	0	1	Kb	?	75.3	Borders
61	9	EP	0	1	Kb	1-6 mths	69.4	Fife
62	9	SH	0	1	Kb	1-6 mths	3.8	Borders
63 [d]	9	AD	0	1	Kb	1-4 wks	48	Borders
64	9	GS	0	1	WC	?	42.8	Borders
65	9	PH	0	1	Kb	1-6 mths	32.3	Borders
66*	10	BC	0	1	WC	6-10 yrs	13.3	Borders
67*	10	GC	1	0	WC	6-10 yrs	10.3	Borders
68	11	DB	1	0	WC	7mths-2 yrs	77.3	Lothian
69	11	SD	0	1	WC	1-7 days	16.7	Lothian
70	11	JD	0	1	WC	1-7 days	44.9	Lothian
71	11	MB	1	0	WC	7mths-2 yrs	37.3	Lothian

patient number	family no	initials	m	f	EBS sub-type	age of onset	age at interview (years)	region
72	12	NH	0	1	Kb	7mths-2 yrs	38.3	Lothian
73	12	DF	0	1	Kb	1-6 mths	41.9	Lothian
74	12	EJF	0	1	Kb	7mths-2 yrs	19.3	Lothian
75	12	DH	0	1	Kb	1-6 mths	7.3	Lothian
76	13	DP	0	1	Kb	birth	39.1	Lothian
77	13	JR	0	1	Kb	birth	19.9	Lothian
78	14	CB	0	1	WC	2-5 yrs	19.7	Borders
79	14	PB	0	1	WC	2-5 yrs	12.8	Borders
80	14	EB	0	1	WC	2-5 yrs	47.6	Borders
81 [d]	14	MG	0	1	WC	2-5 yrs	78.3	Borders
82	15	PM	1	0	Kb	1-6 mths	34.8	Highlands
83	15	CM	0	1	WC	7mths-2 yrs	7.5	Highlands
84	15	DM	1	0	WC	?	33.3	Highlands
85	15	CM	0	1	WC	2-5 yrs	13.9	Highlands
86	15	RM	1	0	WC	6-10 yrs	10.6	Highlands
87*	15	JM	1	0	Kb	?	60	Strathclyde
88	15	RS	0	1	WC	7mths-2 yrs	33	Strathclyde
89	16	JH	1	0	WC	7mths-2 yrs	38.7	Strathclyde
90	16	JH Jnr	1	0	Kb	1-6 mths	13	Strathclyde
91	16	PH	1	0	Kb	1-6 mths	10.8	Strathclyde
92 [d]	16	Jn H	0	1	Kb	7mths-2 yrs	7.7	Strathclyde
93	17	HP	0	1	WC	?	45.7	Borders
94	17	Dv P	1	0	WC	7mths-2 yrs	24.3	Borders
95	17	KD	0	1	Kb	1-4 wks	1.8	Strathclyde
96	18	CMc	0	1	WC	birth	7.9	Strathclyde
97	19	JK	0	1	WC	7mths-2 yrs	29.3	Strathclyde
98	19	MK	0	1	Kb	1-6 mths	1.5	Strathclyde
99	19	GK	1	0	WC	1-6 mths	6.2	Strathclyde
100	19	PS	0	1	WC	7mths-2 yrs	32.8	Strathclyde
101	19	BS	1	0	WC	1-4 wks	14.8	Strathclyde
102	19	CS	1	0	WC	7mths-2 yrs	3.5	Strathclyde
103	19	TS	1	0	WC	?	28	Strathclyde
104	19	MY	0	1	WC	?	35.8	Strathclyde
105	19	AH	1	0	Kb	7mths-2 yrs	11.9	Strathclyde
106*	19	WH	0	1	?	?	40	Strathclyde
107	19	JW	0	1	WC	?	56.3	Strathclyde

patient number	family no	initials	m	f	EBS sub-type	age of onset	age at interview (years)	region
108	20	GH	1	0	Kb	?	55.7	Grampian
109	20	PH	1	0	Kb	1-6 mths	28.6	Grampian
110	20	IM	1	0	Kb	?	57	Highlands
111*	20	MM	0	1	Kb	1-6 mths	31.5	Highlands
112	20	NH	1	0	WC	?	54.8	Highlands
113	20	YA	0	1	Kb	1-7 days	30.4	Highlands
114	21	DL	1	0	Kb	1-7 days	20.1	Strathclyde
115	21	EL	0	1	Kb	1-7 days	24.5	Strathclyde
116	21	CL	0	1	Kb	1-7 days	43.8	Strathclyde
117	21	LL	0	1	Kb	1-6 mths	1.3	Strathclyde
118	21	Dc L	1	0	Kb	1-6 mths	1.5	Strathclyde
119	21	NL	0	1	Kb	1-6 mths	1.5	Strathclyde
120	22	NK	0	1	DM	birth	0.3	Tayside
121 [d]	23	WMc	1	0	WC	?	75.3	Strathclyde
122	24	AMc	1	0	Kb	7mths-2 yrs	7.7	Strathclyde
123	24	MMc	0	1	Kb	7mths-2 yrs	5.5	Strathclyde
124	25	DS	1	0	WC	?	27.3	Tayside
125	25	ES	0	1	WC	1-6 mths	1.3	Tayside
126	26	CMc	0	1	Kb	1-7 days	18.8	Strathclyde
127	27	JMc	0	1	WC	11-20 yrs	20.7	Strathclyde
128*	27	SMc	1	0	WC	11-20 yrs	18.8	Strathclyde
129	28	LD	0	1	Kb	7mths-2 yrs	28.9	Tayside
130 *	28	ED	0	1	Kb	1-6 mths	1.3	Tayside
131	29	AB Jr	1	0	Kb	7mths-2 yrs	9	Strathclyde
132	29	MB	0	1	Kb	1-7 days	5.3	Strathclyde
133 *	29	AB Sr	1	0	WC	7mths-2 yrs	32.3	Strathclyde
134	30	CP	0	1	DM	birth	16.8	Strathclyde
135	31	MS	0	1	DM	1-7 days	48.7	Grampian
136	32	JF	1	0	WC	2-5 yrs	7.5	Strathclyde
137	32	VJF	0	1	WC	11-20 yrs	13	Strathclyde
138*	32	IF	1	0	WC	?	27.1	Strathclyde
139	33	JR	1	0	WC	?	59.9	Strathclyde
140 *	34	GL	1	0	WC	7mths-2 yrs	12.3	Strathclyde
141	35	SZ	1	0	DM	1-7 days	0.1	Strathclyde
142	35	AZ	1	0	DM	1-7 days	0.1	Strathclyde
143*	39	JMc	0	1	WC	?	39	Grampian
144*	40	LI	0	1	WC	?	19	Strathclyde
145*	40	RI	0	1	WC	7mths-2 yrs	0.75	Strathclyde

patient number	family no	initials	m	f	EBS sub-type	age of onset	age at interview (years)	region
146*	41	PMc	1	0	?	1 year	6	Lothian
147*	42	MR	0	1	WC	1 year	7	Strathclyde
148*	42	CR	0	1	WC	?	28	Strathclyde
149*	43	LM	0	1	WC	2 years	15	Ayrshire

* patient not included in chapter 3
d patient died during the study
WC Weber-Cockayne subtype of EBS
Kb Köbner subtype of EBS
DM Dowling-Meara subtype of EBS

Appendix 3

Clinical features and mutations in epidermolysis bullosa simplex

patient number	family number	patient initials	mutation	EBS sub type	hands	feet	other sites	oral mucosa	dystrophic nails	seasonal blisters	winter blisters	hyperkeratosis	improvement	dermatologist	no family history
1	1	JB	K5: Arg331Cys	Kb	1	1	1	0	1	1	1	0	?	1	0
2	1	CB		WC	0	1	0	0	0	1	0	0	?	1	0
3	1	MB		Kb	1	1	1	0	0	1	1	0	?	1	0
4	1	AB		WC	1	1	0	0	0	1	1	0	?	1	0
5	1	DM		WC	0	1	0	0	0	1	1	0	1	0	0
6	1	SM		Kb	0	1	1	0	0	?	0	0	?	0	0
7	2	AM		WC	0	0	0	0	0	0	0	0	1	1	1
8	3	PK	K14:Val270Met	WC	0	1	0	0	0	1	0	0	1	0	0
9	3	KM		Kb	1	1	1	0	1	1	?	0	?	1	0
10	3	KvM		Kb	1	1	1	0	0	1	0	0	?	1	0
11	3	AM		WC	1	1	0	0	0	1	0	0	0	1	0
12	3	LM		Kb	1	1	1	0	0	1	?	0	?	1	0
13	3	RD		Kb	1	1	1	0	0	1	?	0	?	1	0
14	3	Mg W		Kb	1	1	1	0	0	1	?	0	1	1	0
15	3	MW		WC	1	1	0	0	1	1	?	0	0	1	0
16	3	St W		Kb	1	1	1	0	1	1	1	0	0	1	0
17	3	Sn W		Kb	1	1	1	0	1	1	1	0	0	1	0
18	3	HR		Kb	1	1	1	0	0	1	?	0	1	1	0
19	3	AK		Kb	1	1	1	1	0	1	?	1	1	1	0
20	3	LB		WC	0	1	0	0	0	1	1	0	0	0	0
21	3	AB		WC	1	1	0	0	0	1	1	1	1	0	0
22	3	VB		WC	0	1	0	0	0	1	1	0	0	?	0
23	3	GH		Kb	1	1	1	1	0	1	1	1	1	1	0
24	3	Gr H		Kb	1	1	1	0	0	1	?	0	?	1	0
25	3	AH		Kb	1	1	1	1	1	1	1	1	1	1	0
26	3	EH		Kb	0	1	1	0	0	?	1	0	inf	1	0
27	3	RH		Kb	1	1	1	0	1	1	1	1	1	1	0
28	3	SH		Kb	1	1	1	0	0	1	inf	0	inf	1	0
29*	3	ZH		?	?	?	?	?	?	?	inf	?	inf	0	0
30	3	IM		Kb	1	1	1	1	0	1	1	0	1	0	0
31	3	AS		Kb	1	1	1	0	1	0	1	0	1	1	0
32	3	AM		WC	1	1	0	0	1	1	?	0	1	0	0
33*	3	JM		?	0	1	1	0	0	0	0	0	inf	0	0
34	3	GM		WC	1	1	0	0	0	1	0	0	0	0	0

patient number	family number	patient initials	mutation	EBS sub type	hands	feet	other sites	oral mucosa	dystrophic nails	seasonal blisters	winter blisters	hyperkeratosis	improvement	dermatologist	no family history
35	4	KM		WC	1	0	0	0	0	?	?	0	1	1	1
36	4	AM		Kb	1	1	1	0	0	0	1	0	1	1	0
37	5	DC	K14:Arg125His	DM	1	1	1	1	0	?	?	0	1	1	1
38	6	CC	K14:Arg125His	DM	1	1	1	0	1	0	1	0	1	1	1
39	7	AJ	K14:Val270Met	Kb	1	1	1	0	0	1	?	0	?	0	0
40	7	WT		Kb	1	1	1	0	1	1	?	0	?	1	0
41	7	HJ		WC	0	1	0	0	1	1	?	0	0	1	0
42	7	SJ		WC	0	1	0	0	1	1	?	0	0	1	0
43	8	MG	K14:Arg388Cys	WC	0	1	0	0	0	1	?	0		0	0
44	8	MC		WC	0	1	0	0	0	1	?	0	?	0	0
45	8	PC		WC	0	1	0	0	0	1	?	0	?	0	0
46	8	BD		WC	1	1	0	0	0	1	1	0		1	0
47	8	ED		WC	1	1	0	1	0	0	0	0	0		0
48	9	DP	K5:Asn193Lys	Kb	1	1	1	0	0	1	?	0	?	1	0
49	9	EP		Kb	1	1	1	0	0	1	0	0	?	1	0
50	9	NL		Kb	1	1	1	0	0	1	0	0	?	1	0
51	9	DP		Kb	1	1	1	0	0	1	0	0	?	1	0
52	9	RR		Kb	1	1	1	0	0	1	?	0	?	0	0
53	9	BA		Kb	1	1	1	0	0	1	1	0	?	0	0
54	9	CA		Kb	1	1	1	0	0	1	1	0	1	1	0
55	9	VP		Kb	1	1	1	0	0	1	0	0	?	1	0
56	9	BP		WC	1	1	0	0	0	1	?	0	?	1	0
57	9	TH		Kb	0	1	1	0	0	1	?	0	1	1	0
58	9	TH Jnr		Kb	1	1	1	0	0	1	1	0	1	1	0
59	9	CJ		Kb	1	1	1	0	0	1	?	0	1	0	0
60	9	AC		Kb	1	1	1	0	0	1	0	0	1	0	0
61	9	EP		Kb	1	1	1	0	0	1	?	0	1	1	0
62	9	SH		Kb	1	1	1	0	0	1	1	0	?	0	0
63 [d]	9	AD		Kb	1	1	1	0	0	1	?	0	?	0	0
64	9	GS		WC	1	1	0	0	0	1	?	0	?	0	0
65	9	PH		Kb	0	1	1	0	0	1	?	0	?	0	0
66*	10	BC		WC	0	1	0	0	0	1	1	0	?	1	1
67*	10	GC		WC	0	1	0	0	0	1	0	0	?	0	0
68	11	DB	K14:Val133Met	WC	1	1	0	0	0	1	0	0	1	0	0
69	11	SD		WC	1	1	0	0	0	1	0	0	?	1	0
70	11	JD		WC	1	1	0	0	0	1	1	0	?	1	0
71	11	MB		WC	1	1	0	0	0	1	1	0	1	0	0

patient number	family number	patient initials	mutation	EBS sub type	hands	feet	other sites	oral mucosa	dystrophic nails	seasonal blisters	winter blisters	hyperkeratosis	improvement	dermatologist	no family history
72	12	NH	K14:Val133Ala	Kb	1	1	1	0	0	1	1	0	1	1	0
73	12	DF		Kb	1	1	1	1	0	1	1	1	1	0	0
74	12	EJF		Kb	1	1	1	1	0	0	1	1	?	1	0
75	12	DH		Kb	1	1	1	0	0	1	1	0	?	0	0
76	13	DP	not found	Kb	1	1	1	1	0	1	0	0	?	1	1
77	13	JR		Kb	1	1	1	1	0	1	0	0	?	1	0
78	14	CB		WC	1	1	0	0	0	1	?	0	?	0	0
79	14	PB		WC	0	1	0	0	0	1	1	0	?	0	0
80	14	EB		WC	1	1	0	1	0	1	1	0	?	1	0
81[d]	14	MG		WC	0	1	0	0	0	1	?	0	1	1	0
82	15	PM	K5:Asn329Ser	Kb	1	1	1	1	1	1	1	0	0	1	0
83	15	CM	not found	WC	0	1	0	0	0	1	0	0	0	0	0
84	15	DM		WC	1	1	0	1	0	1	?	0	0	0	0
85	15	CM		WC	0	1	0	1	0	1	?	0	0	0	0
86	15	RM		WC	0	1	0	0	0	?	0	0	0	0	0
87*	15	JM	K5: Asp329Ser	Kb	1	1	1	?	0	1	0	?	1	1	0
88	15	RS	not found	WC	0	1	0	0	0	1	?	0	1	0	0
89 [a]	16	JH	K14:Lys116Glu	WC	1	1	0	1	1	1	1	0	1	1	1
90 [a]	16	JH Jnr		Kb	1	1	1	1	0	1	1	0	?	1	0
91 [a]	16	PH		Kb	1	1	1	1	0	1	1	0	?	1	0
92 [a, d]	16	Jn H		Kb	1	1	1	1	0	1	1	0	?	1	0
93	17	HP	K14:Val133Met	WC	1	1	0	0	0	1	1	0	1	0	0
94	17	Dv P		WC	1	1	0	0	0	1	1	0	0	0	0
95	17	KD		Kb	1	1	1	0	0	?	?	0	?	1	0
96	18	CMc		WC	1	1	0	0	0	1	1	0	0	1	1
97	19	JK	K5:Ile467Leu	WC	1	1	0	0	0	1	1	0	?	1	0
98	19	MK		Kb	0	1	1	0	0	1	?	0	inf	1	0
99	19	GK		WC	0	1	0	0	0	1	1	0	0	1	0
100	19	PS		WC	1	1	0	0	0	1	1	0	1	1	0
101	19	BS		WC	1	1	0	0	0	1	1	0	0	1	0
102	19	CS		WC	0	1	0	0	1	1	1	0	?	0	0
103	19	TS		WC	1	1	0	0	0	1	1	1	1	1	0
104	19	MY		WC	1	1	0	0	0	1	1	1	1	1	0
105	19	AH		Kb	1	1	1	0	0	1	0	0	0	1	0
106*	19	WH		?	?	?	?	?	?	?	?	?	?	?	0
107	19	JW		WC	1	1	0	0	0	1	1	1	1	0	0

patient number	family number	patient initials	mutation	EBS sub type	hands	feet	other sites	oral mucosa	dystrophic nails	seasonal blisters	winter blisters	hyperkeratosis	improvement	dermatologist	no family history
108	20	GH	K14:Val133Leu	Kb	1	1	1	0	0	1	1	0	0	1	0
109	20	PH		Kb	1	1	1	0	0	1	1	0	0	1	0
110	20	IM		Kb	1	1	1	0	0	1	1	0	0	1	0
111*	20	MM		Kb	1	1	1	0	1	1	1	0	0	1	0
112	20	NH		WC	1	1	0	0	0	1	1	0	1	1	0
113	20	YA		Kb	1	1	1	1	0	1	1	0	0	1	0
114	21	DL	K14:Tyr129Cys	Kb	1	1	1	0	0	1	1	0	0	1	0
115	21	EL		Kb	1	1	1	0	0	1	1	0	1	1	0
116	21	CL		Kb	1	1	1	0	0	1	1	0	0	1	1
117	21	LL		Kb	1	1	1	0	0	inf	1	0	inf	1	0
118	21	Dc L		Kb	1	1	1	1	0	?	1	0	?	1	0
119	21	NL		Kb	1	1	1	1	1	?	1	0	?	1	0
120	22	NK	K5:Ser181Pro	DM	1	1	1	1	1	inf	1	0	1	1	1
121 [d]	23	WMc		WC	1	1	0	0	1	1	1	0	1	1	1
122	24	AMc		Kb	1	1	1	0	0	1	1	0	0	1	1
123	24	MMc		Kb	1	1	1	0	0	1	1	0	0	1	0
124	25	DS		WC	0	1	0	0	0	0	1	0	?	0	1
125	25	ES		WC	1	1	0	0	0	inf	?	0	inf	1	0
126	26	CMc	K5:Ala428Val	Kb	1	1	1	1	0	1	1	1	?	1	0
127	27	JMc		WC	0	1	0	0	0	1	1	1	?	1	0
128*	27	SMc		WC	0	1	0	0	?	1	?	?	?	1	0
129	28	LD	K5:Glu170Gly	Kb	0	1	1	0	0	1	?	1	1	1	1
130 *	28	ED		Kb	1	1	1	1	1	1	1	0	inf	1	0
131	29	AB Jr	K14:Leu418Val	Kb	1	1	1	0	0	1	1	0	0	1	0
132	29	MB		Kb	1	1	1	1	0	1	1	0	0	1	0
133 *	29	AB Sr		WC	1	1	0	0	?	1	?	?	1	1	0
134	30	CP	K14:Arg125Cys	DM	1	1	1	0	1	0	1	1	1	1	1
135	31	MS	K14:Leu122Ph	DM	1	1	1	0	1	0	1	1	1	1	1
136	32	JF	not found	WC	0	1	0	0	0	1	0	0	0	1	0
137	32	VJF		WC	0	1	0	0	0	1	0	0	0	0	0
138*	32	IF		WC	1	1	0	0	?	1	1	?	?	?	?
139	33	JR	K5:Arg331Cys	WC	1	1	0	0	0	1	1	0	1	1	?
140 *	34	GL	K14:Arg148Cys	WC	0	1	0	0	1	1	1	0	?	0	?
141	35	SZ	K14:Arg125Cys	DM	1	1	1	1	1	?	1	0	1	1	1
142	35	AZ		DM	1	1	1	1	1	?	1	0	1	1	1

patient number	family number	patient initials	mutation	EBS sub type	hands	feet	other sites	oral mucosa	dystrophic nails	seasonal blisters	winter blisters	hyperkeratosis	improvement	dermatologist	no family history
143*	39	JMc	K14:Ile377Thr	WC	1	1	0	0	0	1	1	0	?	1	1
144*	40	LI		WC	1	1	0	1	0	0	0	0	?	?	0
145*	40	RI		WC	1	1	0	1	0	0	0	0	0	0	0
146*	41	PMc		?											
147*	42	MR		WC	1	1	0	0		1	1	0			0
148*	42	CR	blood sent	WC											
159*	43	LM		WC	0	1	0	0	0						

1 feature present
0 feature absent
* patient not included in chapter 3
[d] patient who died during the study
[a] anal fissures present in all members of family 16
inf infant too young for full assessment of clinical features
WC Weber-Cockayne subtype of EBS
Kb Köbner subtype of EBS
DM Dowling-Meara subtype of EBS

Appendix 4

Epidermolysis bullosa simplex patients identified by relatives or other dermatologists

Family number	patient initials	m	f	D.O.B.	Region	Mutation
3	MH	1		25/07/1972	Strathclyde	K14 Val270Met
3	SN		1	10/09/1938	Grampian	
3	KH*	1		20/10/1968	Grampian	
3	SH		1	?	Grampian	
3	MC		1	27/11/1933	Grampian	
3	DN		1	20/10/1968	Grampian	
3	AE	1		21/07/1936	Grampian	
3	JH (Snr)	1		?	Grampian	
3	LH		1	?	Grampian	
3	JH (Jnr)	1		?	Grampian	
11	MD		1	?	?	K14 Val133Met
17	HD		1	?	Strathclyde	K14 Val133Met
19	AM	1		?	Strathclyde	K5:Ile467Leu
19	DS		1	?	Strathclyde	
19	MS		1	01/12/1935	Strathclyde	
24	AMc	1		08/08/1963	Grampian	
26	WMc	1		?	Strathclyde	K5:Ala428Val
27	CB		1	?	Strathclyde	
27	GB	1		?	Strathclyde	
29	MB		1	29/09/1927	Strathclyde	K14:Leu418Val
31	KS	1		14/03/1972	Grampian	K14:Leu122Phe
?	RT	1		03/10/1913	Lothian	
36	CG[d]	1		?	Strathclyde	no tonofilaments but K5 and K14 present [a]
37	JMc[d]		1	29/07/1965	?	
38	AR[d]	1		?	?	
39	FS		1	?	Tayside	K14:Ile377Thr
39	CS	1		?	Tayside	
40	DT	1		04/11/1955	?	
44	DK*		1	27/06/1968	Lothian	blood sent

* patient seen September 2002
a personal communication (E. J. Rugg)
d patient identified by other dermatologists

Appendix 5

Demographic details of dystrophic epidermolysis bullosa patients

patient number	family number	initials	m	f	DEB subtype	age at onset	age at interview (years)	region
1[d]	1	JH	1	0	RDEB-HS	birth	23	Lothian
2	1	PB	1	0	DEB-unc	1-6 months	33	Lothian
3[d]	2	CL	0	1	RDEB-HS (s)	birth	20	Strathclyde
4	3	AH	0	1	RDEB-HS (s)	birth	3.5	Dumph + Gall
5[d]	4	FMcN	0	1	RDEB-inv (s)	1-4 weeks	57	Western Isles
6	5	MMcL	0	1	RDEB-inv (s)	birth	48	Lothian
7	6	SA	0	1	RDEB-unc (s)	birth	6	Lothian
8	7	CC	0	1	RDEB-unc (s)	1-7 days	6	Lothian
9	8	VH	1	0	RDEB	1-4 weeks	43	Lothian
10	9	CY	1	0	DDEB	1-7 days	1.5	Lothian
11	9	SY	1	0	DDEB	?	37	Lothian
12	10	CB	0	1	DDEB	birth	0.04	Lothian
13	10	LB	0	1	DDEB	1-7 days	21	Lothian
14	10	PT	1	0	DDEB	1-6 months	10	Lothian
15	10	LT	0	1	DDEB	?	30	Lothian
16	10	JMcM	0	1	DDEB	?	31	Lothian
17	10	FW	1	0	DDEB	2-5 years	9	Lothian
18	10	DT	0	1	DDEB	7-24 months	3.25	Lothian
19	10	DN	0	1	DDEB	1-6months	5	Lothian
20	10	LN	0	1	DDEB	1-6months	3	Lothian
21	10	VN	0	1	DDEB	1-6months	25	Lothian
22	10	HMcM	0	1	DDEB	?	50	Lothian
23	10	HD	0	1	DDEB	?	27	Lothian
24	10	CD	0	1	DDEB	1-7 days	2	Lothian
25	10	JD	1	0	DDEB	7-24 months	7	Lothian
26	10	MD	1	0	DDEB	7-24 months	9	Lothian
27	10	KD	0	1	DDEB	7-24 months	4	Lothian

patient number	family number	initials	m	f	DEB subtype	age at onset	age at interview (years)	region
28	11	DF	1	0	DDEB	?	65	Lothian
29	11	KF	0	1	DDEB	1-7 days	10	Lothian
30	11	KF	1	0	DDEB	1-7 days	8	Lothian
31	11	DF	1	0	DDEB	1-7 days	40	Lothian
32	11	HD	0	1	DDEB	?	46	Lothian
33	11	HM	0	1	DDEB	birth	37	Lothian
34	11	MR	0	1	DDEB	?	72	Lothian
35	11	JC	0	1	DDEB	1-6 months	4.5	Lothian
36	11	FC	1	0	DDEB	1-6 months	31	Lothian
37	11	AC	0	1	DDEB	?	54	Lothian
38	12	MG	0	1	DDEB	birth	66	Lothian
39	12	GG	1	0	DDEB	?	61	Lothian
40	12	DC	1	0	DDEB	7-24 months	41	Lothian
41	12	MC	0	1	DDEB	?	71	Lothian
42	13	MD	0	1	DDEB	1-7 days	31	Lothian
43	14	GMcK	1	0	DDEB	1-7 days	47	Grampian
44	14	AMcK	0	1	DDEB	1-7 days	11	Grampian
45	14	IMcK	0	1	DDEB	?	76	Grampian
46	15	KMcF	0	1	DEB-unc (s)	1-6 months	6.75	Central
47	16	JC	0	1	DEB-unc (s)	birth	1.75	Strathclyde
48	18	MG	0	1	DEB-unc (s)	1-7 days	14	Strathclyde
49	19	MK	1	0	DEB-unc (s)	1-4 weeks	2	Grampian
50	20	CG	1	0	DDEB	birth	0.08	Strathclyde
51	20	SG	1	0	DDEB	birth	0.08	Strathclyde
52	20	WG	1	0	DDEB	7-24 months	26	Strathclyde
53	20	CG	0	1	DDEB	?	50	Strathclyde
54	21	AG	1	0	RDEB	birth	41	Grampian
55	21	RG	0	1	RDEB	birth	43	Grampian
56	21	DS	0	1	RDEB	birth	36	Highland
57	22	EH	0	1	DDEB	?	51	Strathclyde
58	22	JH	0	1	DDEB	1-6 months	23	Strathclyde
59	22	AK	0	1	DDEB	?	74	Strathclyde
60	23	CC	0	1	DEB-unc (s)	?	39	Grampian
61	24	CA	1	0	DDEB	1-6 months	4	Strathclyde
62	25	KM	0	1	DEB-unc (s)	1-7 days	5	Strathclyde
63	26	DW	1	0	DEB-unc (s)	1-7 days	35	Strathclyde

patient number	family number	initials	m	f	DEB subtype	age at onset	age at interview (years)	region
64	27	NG	0	1	DDEB	7-24 months	31	Strathclyde
65	27	SG	1	0	DDEB	2-5 years	7	Strathclyde
66	27	MG	1	0	DDEB	7-24 months	9	Strathclyde
67	27	JG	1	0	DDEB	?	55	Strathclyde
68	28	MW	1	0	DDEB	1-7 days	28	Strathclyde
69	28	CW	1	0	DDEB	?	35	Strathclyde
70	28	JW	1	0	DDEB	1 yr	7	Strathclyde
71	29	PN	1	0	DEB-unc (s)	1-7 days	33	Strathclyde
72	30	MF	0	1	DDEB	7-24 months	27	Grampian
73	30	IF	0	1	DDEB	?	46	Grampian
74	31	SE	1	0	DEB-unc (s)	1-6 months	15	Dum + Gall
75	32	JM	1	0	DEB-unc (s)	birth	4	Strathclyde
76[dd]	33	EMcG	0	1	RDEB-HS (s)	birth	34	Strathclyde
77	34	MB	1	0	DEB-unc (s)	1-6 months	15	Strathclyde
78	35	CA	0	1	DDEB	1-7 days	50	Fife
79	35	PA	1	0	DDEB	1-7 days	27	Fife
80	35	CA	0	1	DDEB	1- 6months	0.2	Fife
81	36	MS	1	0	DDEB	?	0.1	Grampian
82	36	AS	1	0	DDEB	?	32	Grampian
83	37	AC	1	0	RDEB-HS (s)	1-7 days	0.08	Strathclyde
84	38	DM	1	0	DEB-unc	?	33	Strathclyde
85	39	WM	1	0	RDEB-HS (s)	birth	10	Strathclyde
86	40	JL	0	1	DDEB	2-5 years	32.6	Borders
87	40	AL	0	1	DDEB	2-5 years	3.8	Borders
88	41	WL	1	0	DEB-unc (s)	birth	15	Strathclyde
89	42	SP	0	1	DEB-unc (s)	1-6 months	1.3	Dumph + Gall
90	43	AF	0	1	RDEB-HS (s)	birth	0.01	Tayside
91	44	AF	0	1	DDEB	birth	14.8	Central
92	45	CMcC	0	1	RDEB-HS (s)	birth	0.4	Grampian
93	48	EB	0	1	DEB-unc (s)	2 weeks	0.5	Lothian
94	49	JM	1	0	DDEB	?	61.5	Strathclyde
95	50	SMcC	0	1	DEB-unc (s)	0.25	1.5	Strathclyde
96	51	AB	0	1	DDEB	0.33	2.5	Lothian
97	51	?B	0	1	DDEB	?	>20	Lothian
98	52	LB	1	0	DEB-unc (s)	48 years	66	Strathclyde

patient number	family number	initials	m	f	DEB subtype	age at onset	age at interview (years)	region
99	53	FM	1	0	DDEB	?	2	Lothian
100	53	CM	1	0	DDEB	?	7	Lothian
101	53	?M	0	1	DDEB	?	>20	Lothian

d	patient who died during the study
dd	patient who died during 2002
(S)	sporadic inheritance
Dumph + Gall	Dumphries and Galloway

Appendix 6

Clinical features and mutations in dystrophic epidermolysis bullosa

patient number	family number	initials	DEB subtype	mutation	atrophic albopapuloid	Pasini albopapuloid	blisters/ erosions	scars	milia	ectropion	constipation	anal fissures	oesophageal stricture/ web	dysphagia	oral lesions	dental disease	ankyloglossia	microstomia	syndactyly	contractures	mitten deformity	nail loss	nail dystrophy	otitis externa	seen by dermatologist
1[d]	1	JH	RDEB-HS		0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
2	1	PB	DEB-unc		1	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1
3[d]	2	CL	RDEB-HS (s)		0	0	1	1	1	0	1	0	?	1	1	1	1	1	1	1	1	1	0	0	1
4	3	AH	RDEB-HS (s)		0	0	1	1	1	0	1	1	?	1	1	0	1	1	1	1	0	1	0	1	1
5[d]	4	FMcN	RDEB-inv (s)		0	0	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	1	0	1	1
6	5	MMcL	RDEB-inv (s)	nf	0	0	1	1	?	0	1	1	1	1	1	1	1	1	0	1	0	1	0	1	1
7	6	SA	RDEB-unc		0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	1
8	7	CC	RDEB-unc	nf	0	0	1	1	1	0	1	1	0	?	1	1	0	0	0	0	0	1	1	0	1
9	8	VH	RDEB	nf	0	0	1	1	0	0	1	1	1	1	1	1	0	1	0	1	0	1	1	0	1
10	9	CY	DDEB		0	0	1	1	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
11	9	SY	DDEB		0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
12	10	CB	DDEB		0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
13	10	LB	DDEB		1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
14	10	PT	DDEB		0	0	1	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1
15	10	LT	DDEB		0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1
16	10	JMcM	DDEB		1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
17	10	FW	DDEB		1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
18	10	DT	DDEB		0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
19	10	DN	DDEB		0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
20	10	LN	DDEB		0	0	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0
21	10	VN	DDEB		1	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0
22	10	HMcM	DDEB		0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
23	10	HD	DDEB		1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
24	10	CD	DDEB		0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	10	JD	DDEB		0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	10	MD	DDEB		0	0	1	1	1	0	1	1	?	1	0	0	0	0	0	0	0	0	0	1	0
27	10	KD	DDEB		0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

patient number	family number	initials	DEB subtype	mutation	atrophic albopapuloid	Pasini albopapuloid	blisters/ erosions	scars	milia	ectropion	constipation	anal fissures	oesophageal stricture/ web	dysphagia	oral lesions	dental disease	ankyloglossia	microstomia	syndactyly	contractures	mitten deformity	nail loss	nail dystrophy	otitis externa	seen by dermatologist
28	11	DF	DDEB		1	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	1
29	11	KF	DDEB		1	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1
30	11	KF	DDEB		0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
31	11	DF	DDEB		1	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1
32	11	HD	DDEB	G2043R	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
33	11	HM	DDEB	G2043R	1	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1
34	11	MR	DDEB	G2043R	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1
35	11	JC	DDEB		0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	11	FC	DDEB		1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
37	11	AC	DDEB		0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
38	12	MG	DDEB	G2026R	0	0	1	1	0	0	1	1	0	1	1	1	0	0	0	0	0	0	1	0	1
39	12	GG	DDEB		0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1
40	12	DC	DDEB		1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	1
41	12	MC	DDEB		0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0
42	13	MD	DDEB		0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	1
43	14	GMc	DDEB	G2043R	0	0	1	1	1	0	0	1	?	1	1	1	0	1	0	1	0	1	1	0	1
44	14	AMc	DDEB		0	0	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	1	1	0	1
45	14	IMcK	DDEB		0	0	1	1	0	0	1	0	?	1	0	0	0	0	0	0	0	1	1	0	1
46	15	KMc	DEB-unc (s)		1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
47	16	JC	DEB-unc (s)		0	0	1	1	1	0	1	1	?	1	1	0	0	0	0	0	0	1	0	0	1
48	18	MG	DEB-unc (s)		0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
49	19	MK	DEB-unc (s)		0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
50	20	CG	DDEB		0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
51	20	SG	DDEB		0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
52	20	WG	DDEB		0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
53	20	CG	DDEB		1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0
54	21	AG	RDEB		0	0	1	1	0	0	1	1	1	1	1	1	0	0	0	0	0	1	1	0	1
55	21	RG	RDEB		1	0	1	1	0	0	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1
56	21	DS	RDEB		0	0	1	1	1	0	0	0	1	1	1	1	1	0	0	0	0	0	1	1	1
57	22	EH	DDEB		0	0	1	1	0	0	1	1	?	1	0	1	0	0	0	0	0	0	1	0	1
58	22	JH	DDEB		0	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	1
59	22	AK	DDEB		0	0	1	1	0	0	0	1	?	1	0	0	0	0	0	0	0	0	1	0	0
60	23	CC	DEB-unc (s)	nf	0	0	1	1	1	0	1	1	1	1	0	1	0	0	0	0	0	0	1	0	1

patient number	family number	initials	DEB subtype	mutation	atrophic albopapuloid	Pasini albopapuloid	blisters/ erosions	scars	milia	ectropion	constipation	anal fissures	oesophageal stricture/ web	dysphagia	oral lesions	dental disease	ankyloglossia	microstomia	syndactyly	contractures	mitten deformity	nail loss	nail dystrophy	otitis externa	seen by dermatologist
61	24	CA	DDEB		0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
62	25	KM	DEB-unc (s)		0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	26	DW	DEB-unc (s)		0	0	1	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1	1	1
64	27	NG	DDEB		1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
65	27	SG	DDEB		0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
66	27	MG	DDEB		0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
67	27	JG	DDEB		1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
68	28	MW	DDEB	G2046V	1	0	1	1	0	0	0	0	0	0	1	1	0	1	0	1	0	1	1	1	1
69	28	CW	DDEB		1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	?
70	28	JW	DDEB		0	0	1	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	?
71	29	PN	DEB-unc (s)	nf	1	0	1	1	0	0	0	1	1	1	1	0	1	1	0	1	0	1	1	1	1
72	30	MF	DDEB		0	0	0	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	1	0	1
73	30	IF	DDEB		1	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1
74	31	SE	DEB-unc (s)		1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
75	32	JM	DEB-unc (s)		0	0	1	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1
76[dd]	33	EMc	RDEB-HS		0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
77	34	MB	DEB-unc (s)		1	0	1	1	1	0	1	0	0	1	1	0	0	0	0	0	0	1	1	0	1
78	35	CA	DDEB	G2043R	1	0	1	1	0	0	0	1	?	1	1	1	0	0	0	0	0	1	1	0	1
79	35	PA	DDEB	G2043R	1	0	1	1	1	0	0	1	?	1	1	0	0	0	0	0	0	1	1	1	1
80	35	CA	DDEB		0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
81	36	MS	DDEB		0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
82	36	AS	DDEB		1	0	1	1	1	0	0	0	0	0	0	?	?	?	0	1	0	1	1	?	1
83	37	AC	RDEB-HS	879G→T	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1
84	38	DM	DEB-unc		1	0	1	1	1	0	?	?	0	0	1	?	0	0	0	0	0	?	1	?	1
85	39	WM	RDEB-HS		0	0	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1
86	40	JL	DDEB		0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
87	40	AL	DDEB		0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
88	41	WL	DEB-unc (s)	nf	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?
89	42	SP	DEB-unc (s)		0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?
90	43	AF	RDEB-HS	8697del 11	0	0	1	0	1	0	0	0	0	0	0	inf	0	0	0	0	0	0	1	0	1
91	44	AF	DDEB		0	0	1	1	?	0	0	0	0	0	?	1	0	0	0	0	0	0	1	0	1

patient number	family number	initials	DEB subtype	mutation	atrophic albopapuloid	Pasini albopapuloid	blisters/ erosions	scars	milia	ectropion	constipation	anal fissures	oesophageal stricture/ web	dysphagia	oral lesions	dental disease	ankyloglossia	microstomia	syndactyly	contractures	mitten deformity	nail loss	nail dystrophy	otitis externa	seen by dermatologist
92	45	CMc	RDEB-HS	nf	0	0	1	1	1	0	1	0	0	0	1	inf	0	0	0	0	0	0	1	0	1
93	48	EB	DEB-unc (s)		0	0	1	1	1	0	1	?	0	0	1	inf	0	0	0	0	0	0	0	1	0
94	49	JM	DDEB		0	1	1	1	1	0	0	0	?	1	1	0	0	0	0	0	0	0	1	0	1
95	50	SMc	DEB-unc (s)		0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	?
96	51	AB	DDEB		0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1
97	51	?B	DDEB		0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
98	52	LB	DEB-unc (s)	G1522E	0	0	1	1	1	0	0	?	?	0	0	0	0	0	0	0	0	0	?	?	1
99	53	FM	DDEB		?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
100	53	CM	DDEB		?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
101	53	?M	DDEB		?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?

Abbreviations:

1	feature present
0	feature absent
^{d}	patient who died during the study
^{dd}	patient who died during 2002
nf	DNA analysed but no COL7A1 mutation found
(S)	sporadic inheritance

Appendix 7

Dystrophic epidermolysis bullosa patients identified by relatives or other dermatologists

patient initials	family number	sex	date of birth	region	mutation
LW	10	f	07/05/79	Lothian	
SW	10	f	06/07/81	Lothian	
CS	10	f	?	Lothian	
PS	10	f	?	Lothian	
GS	10	m	09/04/69	Lothian	
MW	10	f	?	Lothian	
JB	12	m	01/01/32	Lothian	
MMcK	14	f	08/01/73	Grampian	
MW [d]	14	f	?	?	
JM *	17	m	09/03/80	Strathclyde	
PAG	20	m	01/01/72	Strathclyde	
PC	20	m	?	Strathclyde	
RE *	21	f	13/05/46	Grampian	
CJ	22	m	?	Strathclyde	
SJ	22	f	15/11/48	Strathclyde	
LMcC	22	m	?	Strathclyde	
MMcC	22	f	?	Strathclyde	
YM	28	f	19/04/40	Strathclyde	G2046V
RF	30	m	?	Grampian	
DS	30	f	?	Grampian	
HL	40	f	?	Borders	
HmcK *	46	f	29/10/97	Grampian	
JG *	47	m	?	Western Isles	
CG *	?	M	24/02/92	Lothian	
RM *	?	m	12/11/65	Lothian	
MW *	?	m	05/04/60	?	
AM *	?49	m	?	Tayside	
ES	?9	f	11/05/34	?	
MV [‡]	24	f		Strathclyde	

* identified by other dermatologists
[d] died during the study
[‡] seen during 2002

Appendix 8
Quality of life questionnaire responses

EBS ≥ 16 years	age (years)	question number and score										total DLQI score
		1	2	3	4	5	6	7	8	9	10	
(n=114)	?	2	1	3	3	2	0	0	2	2	0	15
	?	3	3	3	1	3	3	2	2	2	2	24
	?	3	0	1	3	2	3	3	3	1	1	20
	?	1	0	1	1	1	2	1	0	0	0	7
	?	1	1	1	3	1	0	0	0	0	0	7
	39	3	0	1	1	3	3	2	0	0	1	14
	>40	2	2	2	2	3	3	3	2	1	1	21
	?	2	2	2	2	2	2	2	1	0	2	17
	36	1	3	1	1	1	2	0	0	0	1	10
	?	1	0	0	1	1	0	0	0	0	0	3
	?	2	3	2	3	1	3	0	1	0	2	17
	16	2	1	1	1	1	0	0	1	0	0	7
	?	1	1	0	1	1	0	0	0	0	0	4
	27	0	0	0	0	0	0	0	0	0	0	0
	62	1	0	0	0	0	0	0	0	0	0	1
	25	0	0	0	0	0	0	0	0	0	0	0
	42	1	0	1	1	1	1	1	0	0	0	6
	?	3	0	1	1	1	3	1	1	0	1	12
	23	0	0	0	0	0	0	0	0	0	0	0
	62	0	0	1	0	1	0	0	0	0	0	2
	38	3	2	2	2	2	2	3	2	0	1	19
	?	1	0	0	0	0	0	0	0	0	0	1
	?	2	1	1	1	2	0	0	1	0	1	9
	51	2	2	2	2	3	3	3	1	1	1	20
	?	3	3	1	0	0	0	0	0	0	0	7
	16	3	3	3	3	3	3	3	1	0	3	25
	21	1	1	2	2	1	2	1	0	0	1	11
	39	0	0	0	0	0	0	0	0	0	0	0
	?	2	2	3	1	1	3	2	3	0	1	18
	?	2	1	2	2	2	3	3	0	0	1	16
	32	2	2	2	1	2	3	0	1	3	1	17
	25	2	1	1	0	1	0	1	0	1	1	8
	?	3	0	2	1	2	3	3	1	1	2	18
	?	1	0	1	0	1	0	0	0	0	0	3
	27	2	1	1	2	1	2	0	0	0	1	10
	46	3	2	2	2	2	3	3	2	2	2	23
	21	3	1	3	2	2	0	2	0	0	1	14
	?	2	2	2	2	3	3	1	1	1	1	18
	?	2	3	2	2	3	3	3	3	0	2	23
	?	1	0	1	2	1	1	0	0	0	1	7
	?	1	1	0	2	0	0	1	0	0	1	6
	45	2	3	1	3	2	3	0	0	0	0	14
	71	1	0	1	0	0	0	0	0	0	0	2
	?	1	0	1	1	2	2	0	0	0	0	7
	45	2	2	3	3	3	3	3	3	2	2	26
	63	1	0	0	1	1	1	0	0	0	0	4
	?	0	0	1	1	0	0	0	0	0	1	3

EBS ≥ 16 years (continued)	age (years)	question number and score										total DLQI score
		1	2	3	4	5	6	7	8	9	10	
	?	1	1	1	0	0	2	0	0	0	0	5
	?	3	2	3	2	3	3	3	2	0	2	23
	45	1	0	0	0	0	0	0	0	0	0	1
	?	0	0	0	1	0	0	0	0	0	0	1
	?	2	1	0	2	2	1	0	0	1	1	10
	44	2	0	1	0	2	3	2	0	0	1	11
	?	3	2	2	2	1	0	3	1	0	1	15
	?	1	1	2	2	2	3	0	1	0	1	13
	50	0	0	1	0	1	1	0	0	0	1	4
	?	1	1	1	1	0	2	3	0	0	1	10
number of replies = 57 (50%)												
average	38.9	1.6	1.0	1.3	1.3	1.3	1.5	1.0	0.6	0.3	0.8	10.7
maximum	71	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	26.0
minimum	16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
median	39	2.0	1.0	1.0	1.0	1.0	2.0	0.0	0.0	0.0	1.0	10.0

EBS< 16 years	age (years)	question number and score										total CDLQI score
		1	2	3	4	5	6	7	8	9	10	
(n=29)	1	3	0	0	3	3	3	2	0	3	2	**19**
	?	2	2	2	1	1	0	2	1	1	3	**15**
	15	1	0	0	0	1	0	0	0	0	0	**2**
	12	3	3	3	3	3	3	2	3	3	3	**29**
	15	3	3	2	3	3	2	3	1	3	2	**25**
	15	3	3	3	3	3	3	3	3	3	3	**30**
	0.9	2	0	0	2	0	0	0	0	1	1	**6**
	?	3	2	1	2	2	3	2	2	2	2	**21**
	?	2	1	3	2	3	3	3	0	0	0	**17**
	4	2	1	0	3	2	1	1	0	3	2	**15**
	4	2	1	0	2	2	0	1	0	2	2	**12**
	7	2	1	0	2	3	1	2	0	2	2	**15**
	8.5	0	0	0	0	0	0	0	0	0	0	**0**
	?	2	1	2	1	2	2	1	1	2	1	**15**
	?	3	3	3	1	3	3	2	2	2	2	**24**
	4	2	2	0	2	2	1	1	1	1	1	**13**
	13	2	1	0	1	2	1	1	0	0	2	**10**
	?	1	1	0	0	0	1	1	2	1	0	**7**
number of replies =18												
average	8.3	2.1	1.4	1.1	1.7	1.9	1.5	1.5	0.9	1.6	1.6	**15.3**
maximum	15	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	**30.0**
minimum	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
median	7.75	2.0	1.0	0.0	2.0	2.0	1.0	1.5	0.5	2.0	2.0	**15.0**

DDEB ≥ 16 years	age	question number and score										total DLQI score
		1	2	3	4	5	6	7	8	9	10	
(n=71)	?	1	2	1	0	0	0	1	0	1	0	**6**
	?	3	3	1	2	2	3	1	1	0	1	**17**
	84	0	0	0	0	0	0	0	0	0	0	**0**
	56	1	0	0	0	0	0	0	0	0	1	**2**
	56	1	0	0	0	0	0	1	0	0	1	**3**
	80	0	0	0	0	0	0	0	0	0	0	**0**
	66	1	0	0	2	0	0	0	0	0	0	**3**
	?	0	0	0	1	0	1	0	0	0	0	**2**
	56	3	3	3	3	3	3	3	1	3	3	**28**
	?	2	1	2	2	3	3	2	1	2	2	**20**
	?	0	1	0	0	0	0	0	0	0	0	**1**
	34	0	0	1	0	0	0	0	0	0	0	**1**
	?	2	2	2	1	2	2	3	1	1	2	**18**
	?	1	0	0	1	1	0	0	0	0	1	**4**
	51	3	1	1	1	1	3	1	1	0	1	**13**
	18	1	1	0	1	0	0	0	0	0	0	**3**
	67	0	0	0	0	0	0	0	0	0	0	**0**
	?	0	0	0	1	0	0	0	0	0	0	**1**
	18	0	0	0	0	0	0	0	0	3	0	**3**
	?	2	1	1	1	1	2	3	1	1	1	**14**
	?	2	2	0	3	2	0	1	1	1	1	**13**
	?	0	0	0	0	0	0	0	0	0	0	**0**
	40	3	2	2	3	2	0	0	1	0	3	**16**
	16	1	1	0	1	1	0	1	0	0	1	**6**
	?	1	2	0	2	0	0	1	0	0	0	**6**
	?	1	1	0	1	0	0	0	0	1	0	**4**
	?	0	0	0	0	0	0	0	0	0	0	**0**
	63	1	1	0	1	1	0	0	0	0	0	**4**
	?	2	3	1	2	2	3	2	1	1	1	**18**
	44	2	3	0	1	2	3	0	3	1	2	**17**
	?	2	1	0	1	0	0	1	0	0	1	**6**
	28	1	1	1	2	0	3	1	0	1	1	**11**
number of replies = 32 (45%)												
average	48.6	1.2	1.0	0.5	1.0	0.7	0.8	0.7	0.4	0.5	0.7	**7.5**
maximum	84	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	**28.0**
minimum	16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
median	53.5	1.0	1.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.5	**4.0**

DDEB < 16 years	age	question number and score										total CDLQI score
		1	2	3	4	5	6	7	8	9	10	
(n=28)	6	3	2	1	2	2	1	1	1	2	2	**17**
	?	3	0	0	2	0	2	0	0	3	2	**12**
	8	0	0	0	0	0	0	0	0	0	0	**0**
	?	3	3	1	2	2	0	2	3	1	2	**19**
	8	0	1	0	0	0	0	1	1	0	1	**4**
	8	0	1	0	0	0	0	1	1	0	1	**4**
	14	2	2	0	2	2	2	1	0	1	1	**13**
	15	2	3	2	3	1	3	2	3	2	2	**23**
number of replies = 8 (29%)												
average	9.8	1.6	1.7	0.5	1.4	0.9	1.0	1.0	1.1	1.1	1.4	**11.5**
maximum	15	3	3	2	3	2	3	2	3	3	2	**23**
minimum	6	0	0	0	0	0	0	0	0	0	0	**0**
median	8	2	2	0	2	0.5	0.5	1	1	1	1.5	**12.5**

RDEB-HS >16 years	age	question number and score										total DLQI score
		1	2	3	4	5	6	7	8	9	10	
(n=1)	40	3	3	0	3	3	0	0	3	0	3	**18**

RDEB-HS <16 years	age	question number and score										total CDLQI score
		1	2	3	4	5	6	7	8	9	10	
(n=5)	3.5	3	3	3	3	3	3	3	2	3	3	**29**
	3.5	3	2	0	2	2	3	1	0	2	2	**17**
	3.5	3	3	0	2	2	3	2	1	3	3	**22**
	?	2	2	1	2	3	3	0	2	2	3	**20**
number of replies = 4 (80%)												
average	3.5	2.8	2.5	1.0	2.3	2.5	3.0	1.5	1.3	2.5	2.8	**22.0**
maximum	3.5	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	3.0	3.0	**29.0**
minimum	3.5	2.0	2.0	0.0	2.0	2.0	3.0	0.0	0.0	2.0	2.0	**17.0**
median	3.5	3.0	2.5	0.5	2.0	2.5	3.0	1.5	1.5	2.5	3.0	**21.0**

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Associated published papers

1. The prevalence of epidermolysis bullosa in Scotland.
   Horn HM, Priestley GC, Eady RAJ, Tidman MJ. Br J Dermatol 1997;136:560-564.

2. A recurrent glycine substitution mutation, G2043R, in the type VII collagen gene (COL7A1) in dominant dystrophic epidermolysis bullosa.
   Mellerio JE, Salas-Alanis JC, Talamantes ML, Horn HM, Tidman MJ, Ashton GHS, Eady RAJ, McGrath JA. Br J Dermatol 1998;139:730-737.

3. Pyloric atresia-junctional epidermolysis bullosa syndrome: mutations in the β4 gene (ITGB4) in two unrelated patients with mild disease.
   Mellerio JE, Pulkkinen L, McMillan JR, Lake BD, Horn HM, Tidman MJ, Harper JI, McGrath JA, Uitto J, Eady RAJ.

4. Laryngeal involvement in the Dowling-Meara variant of epidermolysis bullosa simplex with keratin mutations of severely disruptive potential.
   Shemanko CS, Horn HM, Keohane SG, Hepburn N, Kerr AIG, Atherton DJ, Tidman MJ, Lane EB. Br J Dermatol 2000;142:315-320.

5. The clinical spectrum of epidermolysis bullosa simplex.
   Horn HM, Tidman MJ. Br J Dermatol 2000;142:468-472.

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The prevalence of epidermolysis bullosa in Scotland

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Accepted for publication 18 October 1996

Summary The prevalence of epidermolysis bullosa (EB) in Britain and most other countries is unknown. Patients suffering from the inherited forms of EB and living in Scotland have been traced. Two hundred and fifty-nine affected people from 76 families have been identified, of whom 211 were clinically assessed. One-third of these Scottish EB sufferers had never been seen by a dermatologist. In Lothian, where there appears to be a relatively high prevalence of EB, 75% of patients were unknown to their general practitioners.

The point prevalence of all forms of EB at the outset of the study was 49·0 per million, comprising EB simplex 28·6 per million and dystrophic EB 20·4 per million. Extrapolation of accurate data available for the Lothians suggests that the point prevalence of all forms of EB in Scotland is in excess of these figures.

There are few reliable studies of the epidemiology of the inherited forms of epidermolysis bullosa (EB) (Table 1). The most detailed surveys have been undertaken in Norway, Northern Ireland and Finland,[1–3] each with a population of less than 5 million. The prevalence of EB has been assessed in several other countries,[4–8] but the low figures obtained suggest either incomplete sampling or pronounced regional variation.

A national register of EB sufferers in Britain is being compiled under the aegis of the Dystrophic Epidermolysis Bullosa Research Association (DEBRA) with the aim of yielding accurate information on the number of affected individuals and the impact of this group of disorders on the lives of sufferers and their families. It will provide data on the medical and social requirements of those affected. Similar EB registers are being compiled in the U.S.A. and Italy.[9,10]

As part of the United Kingdom EB Register, Scotland was selected as a politically and geographically well defined area (population 4,998,567[11]), for intensive assessment of those suffering from EB. This paper reports on the prevalence of EB.

Methods

Scottish patients suffering from inherited forms of EB were identified from a diagnostic index at the Royal Infirmary of Edinburgh, and by general practitioners and Scottish dermatologists. All 515 Lothian general practitioners and all 38 Scottish dermatology consultants were asked to provide, with patient consent, details of any EB sufferers known to them. An advertisement directed at people suffering from blisters was placed by DEBRA in a newspaper distributed in rural western Scotland. Further patients were identified by family enquiries. All known affected individuals were invited to participate in the survey. Patients were interviewed and examined in their own homes or at the Royal Infirmary of Edinburgh. Parents and siblings were also examined whenever possible. If personal contact was not feasible, patients were interviewed by telephone.

Interviews were conducted with the help of a detailed questionnaire and answers were entered on to a computerized database. Classification of EB subtype was made on the basis of clinical features and, where appropriate, ultrastructural examination, using the criteria suggested by Fine *et al.*[12] Patients were classified as 'sporadic' if there was no family history of EB. Details of all known EB patients living in Scotland between May 1992 and March 1996 are included in this paper.

Results

Two hundred and fifty-nine patients from 76 families were identified as suffering from EB (Table 2). Their geographical distribution is shown in Fig. 1. Enquiries to all 515 general practitioners in Lothian (population

Table 1. Reported prevalences of epidermolysis bullosa (EB) (per million)

	EBS	JEB	DEB
Norway[1]	24·3	–	9·3
N. Ireland[2]	28	0·7	3·3
Finland[3]	15·1	0·2	8·8
Croatia[4]	1·5	1·5	6·6
Japan[5]	4·0	0·2	3·5
Saudi Arabia[6]	1·7	0	3·7
S. Africa[7]	0·8	0·7	1·2

EBS, EB simplex; JEB, junctional EB; DEB, dystrophic EB.

726,010[11]) brought replies from 340 doctors representing 93% of practices. They were aware of only 20 of the 78 documented Lothian EB patients and also notified us of one previously unidentified sufferer who would subsequently have been found by family enquiries. Of the 29 (76%) Scottish dermatologists who replied to our enquiries, 13 were aware of 37 EB patients. Fifteen of these individuals from 12 families were previously unknown to us. The newspaper advertisement did not elicit any replies. One hundred and fifty-seven patients were identified by family enquiries or were referred to us routinely during the study.

Of the 259 individuals identified as suffering from EB, 211 (81%) were clinically assessed. Twelve interviewed subjects were not examined, information being obtained either by telephone, or from a parent, spouse or sibling. Thirty-six patients (20 EB simplex sufferers and 16 dystrophic EB sufferers), all relatives of people in whom the diagnosis and subtype of EB was confirmed, were, for practical reasons, neither interviewed nor clinically assessed.

Epidermolysis bullosa simplex

EB simplex (EBS) was diagnosed in 149 individuals (58 male, 91 female) from 33 families. One hundred and twenty-five patients from 30 families were interviewed

Table 2. Numbers of Scottish individuals and families identified as suffering from epidermolysis bullosa (EB)

	EBS	JEB	DEB	Total
Numbers of individuals	149	2	108	259
Numbers of families	33	2	41	76

EBS, EB simplex; JEB, junctional EB; DEB, dystrophic EB.

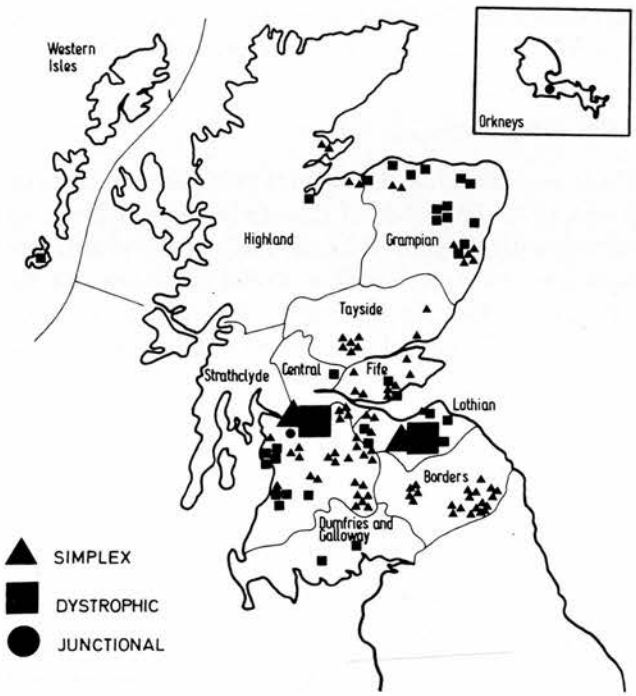

Figure 1. Geographical distribution of epidermolysis bullosa in Scotland. Small symbols represent individuals; large symbols represent multiple individuals within a small area.

and 121 patients from 33 families were examined clinically.

Four subjects (one male, three female), each of whom were sporadic cases, had the Dowling–Meara (DM) variant. Their ages at interview ranged from 1 month to 16·7 years (average 5·2 years). Electron microscopic examination of skin from all four showed the characteristic ultrastructural features of this condition.[13]

The remaining 121 interviewed EBS sufferers, aged from 1·3 years to 78·3 years (average 30·2 years), were from 26 pedigrees, one with 36 and a second with 19 living affected individuals. Ten patients had no family history of the disorder. Fifty experienced blisters on only the palms and soles, as described originally by Weber and Cockayne,[14,15] and 71, while also developing blisters predominantly on the palms and soles, experienced blisters at additional friction sites, thus fitting Koebner's original description of EBS.[16] Other variants of EBS were not seen.[12]

Thirty-eight (30%) of the EBS patients interviewed had never consulted a dermatologist. One 9-year-old girl with Koebner EBS died from meningitis during the study.

The point prevalence of EBS on 1 May 1992 is calculated as 28·6 per million, with the DM subtype

accounting for 0·6 per million. The incidence of EBS is at least 0·43 per million per year, over 46 years.

Junctional epidermolysis bullosa

There were no known junctional EB (JEB) sufferers in Scotland at the outset of the study. Two males were subsequently diagnosed on the basis of the ultrastructural features and reduced or absent expression of GB3 antigen. The first, born to apparently unrelated parents, had a relatively mild phenotype, but the second, born in Libya to healthy Libyan parents who were first cousins, had the Herlitz (letalis) subtype of JEB. Both children were still alive at the conclusion of the study. The incidence of JEB over 4 years was 0·05 per million per year.

Dystrophic epidermolysis bullosa

Dystrophic EB (DEB) was identified in 108 individuals from 41 families; 88 patients from all 41 families were clinically assessed and 85 patients from 37 families were formally interviewed.

Four subtypes of DEB were seen. Localized dominant DEB (DDEB) occurred in 78 individuals (35 male, 43 female) from 14 families and 59 of these patients were interviewed. Their ages at interview ranged from 1 month to 76·5 years (average 28·9 years). Localized recessive DEB (LRDEB) was seen in five patients (two male, three female) from two families. They were aged between 36 and 48 years (average age 42·4 years). Two unrelated females, aged 47 years and 56 years suffered from the inverse variant of recessive DEB (RDEB-inv). The elder has recently died (aged 60 years) from a squamous cell carcinoma of the oesophagus. The severe generalized Hallopeau–Siemens form of recessive DEB (RDEB-HS) was seen in five unrelated patients (two males, three females), aged 1 month to 34·5 years (average age 16·2 years). Two died during the study period, one (aged 23 years) from metastatic squamous cell carcinoma and the second (aged 20 years) from gastric aspiration during a 'flu-like' illness. Eighteen patients with localized DEB (11 male, seven female) aged 2 months to 43·1 years (average age 16·1 years) had unaffected parents and siblings and their inheritance pattern could not be accurately classified.

The parents of one patient with RDEB-inv were shown to share a common ancestor, but consanguinity was not known in any other families affected by DEB. One patient with mild localized DEB but unaffected parents and siblings had a cousin with severe RDEB-HS, as previously reported by Kemmett *et al.*[17]

Twenty-four (30%) interviewed DDEB patients had never been seen by a dermatologist. The point prevalence, on 1 May 1992 of all forms of DEB is calculated as 20·4 per million (DDEB 14·6, LRDEB 1·0, RDEB-inv 0·4, RDEB-HS 0·8, sporadic localized DEB 3·6 per million) and the incidence of DEB since 1950 is at least 0·2 per million per year.

Discussion

Accurate estimates of the prevalence of EB are difficult to achieve. While the most severely afflicted EB sufferers are well known to the medical profession, we have shown that a large proportion of sufferers of the milder forms of EB are unknown to both general practitioners and dermatologists; 30% of patients with both EBS and DDEB in Scotland had never been seen by a dermatologist. Although these patients do not have life threatening disease, most, particularly those with EBS, have blistering which significantly restricts their lives. It emerged from interviews that many felt, based on the experiences of similarly afflicted relatives, that there was little to be gained from consulting a doctor. Some patients had suffered at the hands of well-meaning but uninformed members of the medical profession who had inflicted considerable pain by using unsuitable dressings. Predictably, such people are reluctant to subject their children to similar trauma and many prefer to use long established family remedies.

Comparison of the prevalence of EB in Scotland with other countries (Tables 1–3) shows figures for EBS similar to those of Norway and Northern Ireland. The relatively high Scottish figure for DEB is accounted for by the large group suffering from DDEB. It is unlikely (though possible) that the prevalence of this often mild subtype is higher in Scotland than elsewhere; rather it is probable that many of these patients go unrecognized.

Table 3. Epidermolysis bullosa (EB) in Scotland

	EBS	JEB	DEB
Point prevalence on 1/5/92 ($\times 10^{-6}$)	28·6	0	20·4
Period prevalence ($\times 10^{-6}$) (1/5/92–1/5/96)	29·8	0·4	21·6
Incidence ($\times 10^{-6}$)	0·43	0·05	0·2

EBS, EB simplex; JEB, junctional EB; DEB, dystrophic EB.

Table 4. Point prevalences on 1 May 1992 of epidermolysis bullosa (EB) in Scottish regions ($\times 10^{-6}$)

	EBS	DDEB	A11 DEB	A11 EB
Borders	240·6	0	0	240·6
Central	0	0	3·7	3·7
Dumfries and Galloway	0	0	13·5	13·5
Fife	23·4	5·8	5·8	29·2
Grampian	27·7	17·8	27·7	55·4
Highland	19·6	0	4·9	24·5
Lothian	42·6	55·0	64·7	107·3
Strathclyde	22·6	9·7	14·6	37·2
Tayside	18·2	0	2·6	20·8
Orkney	0	0	0	0
Western Isles	0	0	33·7	33·7
Shetlands	0	0	0	0
Scotland	28·6	14·6	20·4	49·0

EBS, EB simplex; DDEB, localized dominant EB; DEB, dystrophic EB.

They will not be found unless all family members are clinically examined. This condition can be so mild that the patient may regard his skin as normal and the physical signs may be very subtle.

The most ambitious EB register being compiled to date is in the U.S.A. It is not yet complete but have data on 1750 patients.[9] As in Scotland, just over 50% of their patients have EBS, but 8·1% (compared with 0·1% in Scotland) suffer from JEB and 15·6% (compared with 4·7% in Scotland) suffer from the various RDEB subtypes. Only 12·4% of their patients suffer from DDEB, while this subtype accounts for 30·2% of our study population. Some of these differences, probably reflect the relative ease with which the most severely affected people are traced, and will become less marked as people with milder EB are included in the American register. If the prevalence of EB is similar in the U.S.A. to that in Scotland then they can expect to find between 11,000 and 12,000 EB sufferers.

The prevalences of the recessive subtypes of EB in Scotland are similar to those reported from other countries, with the exception of Croatia where there appears to be a surprisingly high prevalence of RDEB-HS.[4] We have found a high prevalence of EBS in the Borders Region (Table 4), where there are four apparently unrelated pedigrees, one having 19 affected living members. This is a rural area with a low population density, and although the families do not knowingly share a common ancestor, this remains a possibility. A mutation of the keratin 5 gene has been identified in the largest family[18] and results of DNA analysis from a second family are awaited.

In other rural areas of Scotland, the prevalence of EB is consistently lower than in regions where the major cities are located (Table 4). Perhaps EB sufferers gravitate towards major centres to avail themselves of medical services and opportunities for non-manual employment.

The prevalence of EB in Lothian is particularly high (EBS 42·6 per million, DEB 64·7 per million), compared with other areas of Scotland. This is probably accounted for by more complete searching on our home territory where we have the benefit of an EB database and where our interest is known to local general practitioners. It seems reasonable to assume that the prevalence of EB in other urban areas of Scotland should be similar to that in Lothian, and if this is so, many Scottish EB patients distant from Edinburgh remain undetected. Our figures are undoubtedly underestimates. Since the study was completed, more previously unknown patients have come to light. This suggests that EB is a more common condition than was hitherto thought.

Acknowledgments

We are grateful to all the dermatologists and general practitioners who helped identify patients suffering from EB, and to DEBRA for funding this work.

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FAST-TRACK PAPER

A recurrent glycine substitution mutation, G2043R, in the type VII collagen gene (COL7A1) in dominant dystrophic epidermolysis bullosa

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Accepted for publication 25 May 1998

Summary Dystrophic epidermolysis bullosa (DEB) is caused by mutations in the type VII collagen gene (COL7A1). Nearly all cases of dominant DEB are caused by glycine substitution mutations occurring within the triple helical region of type VII collagen, and most of the mutations are unique to individual families. In this study, we identified a patient of Hispanic-Mexican origin with a mild form of DEB, which resulted from a *de novo* dominant glycine substitution, G2043R, in exon 73 of COL7A1. We also investigated a Scottish family with a three-generation pedigree of dominant DEB, in whom the same glycine to arginine substitution mutation was demonstrated. This particular mutation has also been detected previously in three other families with dominant DEB: one Italian, one Hungarian and one Norwegian. Given the widespread geographical distribution of this mutation and the demonstration of its occurrence as a *de novo* event, G2043R therefore represents the first example of a mutational hotspot in dominant DEB. Interestingly, although both the Mexican and Scottish families we studied had some clinical features in keeping with the Pasini form of the disorder, there was considerable interfamilial variability as well as intrafamilial diversity in the affected individuals.

Both autosomal dominant and autosomal recessive forms of the inherited blistering skin disorder, dystrophic epidermolysis bullosa (DEB), are caused by mutations in the type VII collagen gene (COL7A1).[1–5] Mutations in dominant subtypes of DEB usually comprise glycine substitutions in the triple helix of COL7A1[6–8] and such mutations may impede the normal secretion of type VII collagen from basal keratinocytes into the extracellular space, disrupt anchoring fibril assembly at the dermal–epidermal junction and lead to a sublamina densa plane of tissue fragility (Fig. 1).[9–13]

Several different glycine substitution mutations resulting in dominant DEB have been described, most of which are unique to individual families.[14–27] In this study we investigated the molecular basis of DEB in two affected families, one from Mexico and one from Scotland, using polymerase chain reaction (PCR) amplification of genomic DNA, heteroduplex analysis, direct automated sequencing of PCR products displaying heteroduplex bandshifts and verification of mutations by restriction endonuclease digestion. In the Mexican family, we identified a patient with a mild form of DEB and clinically normal parents, who had a *de novo* glycine substitution, G2043R, in exon 73 of COL7A1. In the unrelated family from Scotland, we delineated the same mutation as the cause of dominant DEB in three generations. The mutation G2043R has been described previously in one Italian, one Hungarian and one Norwegian family, all with dominant DEB.[17,22,23] Thus, this mutation represents the first recurrent hotspot mutation in the autosomal dominant form of this genodermatosis.

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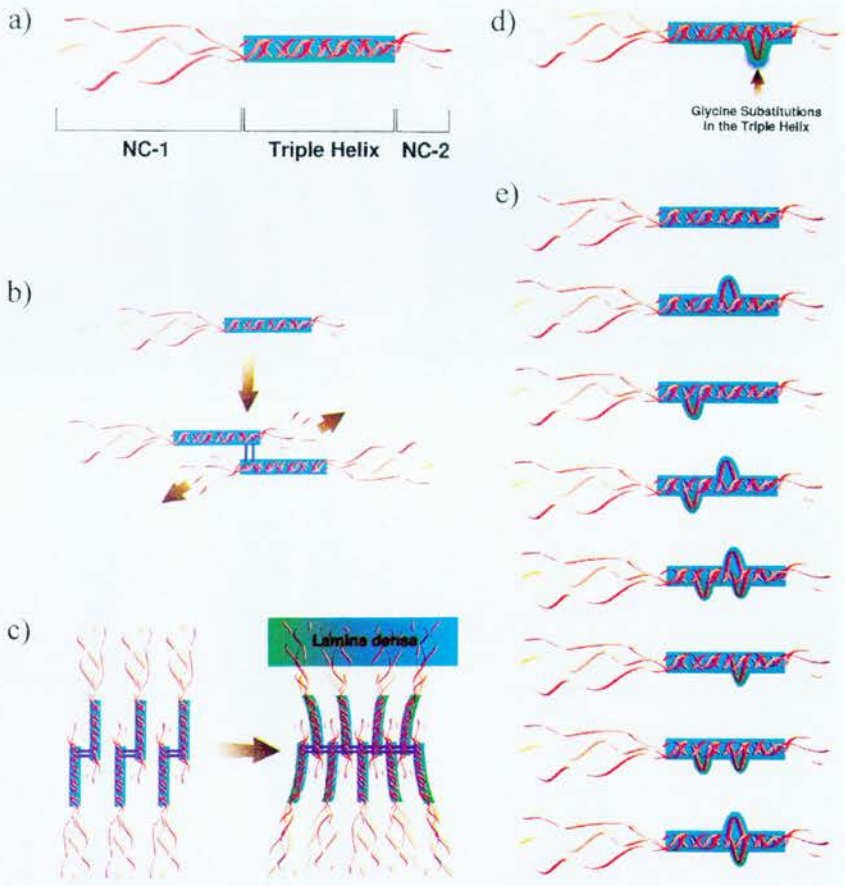

Figure 1. Diagrammatic illustration of anchoring fibril assembly and pathology underlying dominant dystrophic epidermolysis bullosa (DEB). (a) Type VII collagen comprises a homotrimer, with a large non-collagenous NC-1 domain, a central collagenous triple helix and a smaller non-collagenous NC-2 domain. The homotrimer is synthesized within basal keratinocytes (and fibroblasts) and secreted into the extracellular space. (b) Two homotrimers then align to form an antiparallel dimer, with disulphide bond formation and cleavage of part of the NC-2 domain. (c) Several antiparallel dimers then aggregate laterally to form anchoring fibrils, which insert into the lamina densa, have a fan-shaped appearance and demonstrate central cross-banding. (d) In dominant DEB, the mutations comprise glycine substitutions within the collagenous triple helix, which disrupt the tightly coiled formation of the helix. (e) The homotrimers may contain none, one, two or three abnormal type VII procollagen chains. Some or several of these polypeptides may not be secreted into the extracellular space. For protein that is secreted, lateral aggregation of dimers may be compromised with consequent disruption of anchoring fibril morphology. Assuming equal expression of wild-type and mutant alleles, and similar secretion of wild-type and mutant protein, only one in 64 antiparallel dimers would be expected to have a normal configuration.

Materials and methods

Patient details

Pedigrees of the two families investigated are shown in Fig. 2. In family A (Fig. 3), the proband was a 25-year-old Hispanic-Mexican woman with a lifelong history of trauma-induced skin fragility. None of her 13 siblings had any history of blisters or erosions. Similarly, neither of her non-consanguineous parents had any skin or nail abnormalities. Examination showed blisters, erosions and scarring, particularly over bony prominences. Nail dystrophy and loss of some fingernails and toenails was also evident. In addition, numerous albopapuloid lesions were detected on the trunk.

In family B (Figs 4 and 5), there were three affected individuals in three generations. Individual II-2 (Fig. 4), a 31-year-old white caucasian, had a history of trauma-induced blisters since childhood, with thickening of nails and loss of some nail plates. As an adult, the blistering tendency had been less marked with the main feature being minor scarring over the bony prominences. His daughter (Fig. 5), aged 3 years, had

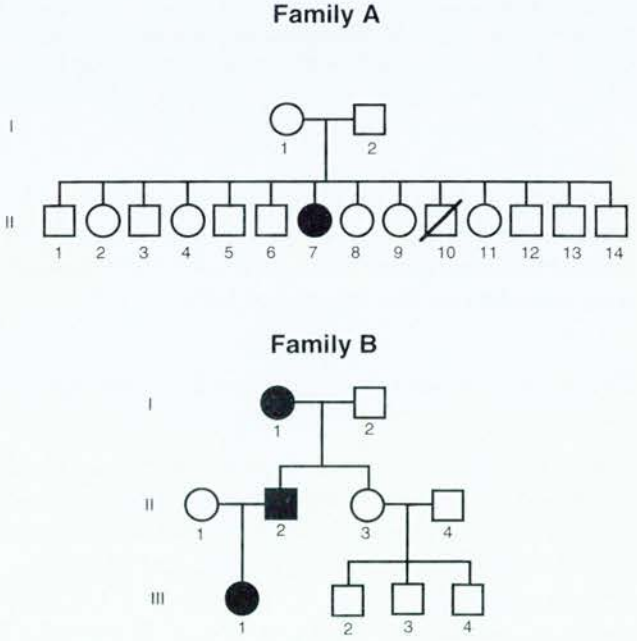

Figure 2. Pedigrees of the two families studied. Family A is from Mexico and contains one affected individual (II-7) who has clinically normal parents. Family B is from Scotland and has three affected family members (I-1, II-2 and III-1) in three generations.

Figure 3. Clinical appearance of the affected individual (II-7) in family A. (a) Fingernails are thickened and dystrophic with loss of nail plates on some fingers. (b) There is marked scarring and blistering over both elbows. (c) Scarring and postinflammatory hyperpigmentation are present on both knees. (d) Most of the toenails have been lost and there is moderate scarring around the ankles. (e) Numerous albopapuloid lesions are present on the back.

numerous recurrent blisters and milia induced by trauma, particularly on the knees and around the ankles. Nail dystrophy was not a prominant feature, with only slight thickening of one great toenail.

Polymerase chain reaction amplification and heteroduplex analysis

Genomic DNA was extracted from peripheral blood lymphocytes using standard methods[28] and was used as a template for amplification of genomic sequences of COL7A1 (GenBank L23982, L02870). Pairs of oligonucleotide primers spanning all 118 exons of the gene were synthesized on the basis of intronic sequences to generate PCR products.[29,30] Specifically, to amplify exons 73 and 74 (see results), the following primers were used: sense primer 5′-CCCGTGGAGTGGGGTGTAGC-3′; antisense primer 5′-TGCAGGAAACAAGAAAATGG-3′. The expected PCR product size was 469 bp. For PCR amplification, approximately 250 ng of genomic DNA was used as the template in an amplification buffer containing 6·25 pmol of the primers, 37·5 nmol $MgCl_2$, 5 mmol of each nucleotide and 1·25 U *Taq* polymerase (Perkin Elmer, Warrington, U.K.) in a 25-μL total volume reaction in an OmniGene thermal cycler (Hybaid, Teddington, U.K.). The amplification conditions were 94 °C for 5 min; then 94 °C for 45 s, 55 °C for 45 s, 72 °C for 45 s, for 38 cycles. Aliquots of 5 μL of the PCR products were analysed by 2% agarose gel electrophoresis, and 3–8 μL of the sample were prepared for heteroduplex analysis using conformation-sensitive gel electrophoresis.[31] Staining with ethidium bromide was used to visualize the heteroduplexes. PCR products displaying altered electrophoretic patterns

Figure 4. Clinical appearance of one of the affected individuals (II-2) in family B. (a) Nail dystrophy is present in all fingernails and there are scattered scars on the dorsum of the hands and fingers. (b) Toenails are either thickened or have been shed, but there is little evidence of scarring on the lower legs or feet. (c) A small degree of crusting and scarring is seen on both elbows. (d) Subtle scarring is present on the knees with no signs of active inflammation. (e) Minor scarring is present on the heel at a site of previous trauma.

were sequenced directly using dye terminator labelling in an ABI 310 genetic analyser (Perkin Elmer) and mutations detected in the PCR products (see results) were verified in genomic DNA by restriction endonuclease digestion. Digestions were performed according to the manufacturer's instructions (New England BioLabs, Hitchin, U.K.).

Results

Molecular analysis

In family A, heteroduplex analysis of PCR products spanning exons 73/74 revealed heteroduplex bands in the affected individual's sample, but only a homoduplex band in the parents' samples and in the control PCR product (Fig. 6). In family B, similar bandshifts were present in DNA from the two affected individuals examined (I-1 and II-2), but these were not detected in unaffected family members (I-2 and II-3) or in a further sample of amplified control DNA. Automated nucleotide sequencing of PCR products from individuals displaying bandshifts (II-7 in family A and II-2 in family B) revealed a heterozygous point mutation in both cases, a G-to-A substitution at nucleotide position 6127 (GenBank L02870) that converts a glycine residue (GGG) to arginine (AGG), the mutation being designated G2043R. This mutation results in loss of a *Sma*I restriction site (CCC/GGG) which was used to assess the presence or absence of the mutation in all members of the two families from whom DNA samples were available. In control DNA and all unaffected individuals, the 469 bp PCR product was digested into fragments 264, 160 and 45 bp in size, while in the affected family members, an additional undigested band of 309 bp was present, indicating that these individuals were all heterozygous for the mutation G2043R in exon 73 of COL7A1. Of note, in family A, the mutation G2043R was not detected in

Figure 5. Clinical appearance of one of the affected individuals (III-1) in family B (daughter of II-2 depicted in Figure 4). (a) There is no significant fingernail dystrophy, but prominent milia and scarring are present over the dorsum of the interphalangeal joints. (b) Similar changes are present in the toes, although slight dystrophy of the great toenail is also present. (c) On the knee there is prominent inflammation with focal erosions and surface crusting. (d) Tense inflammatory blisters are noted on and around the ankle.

either parent's DNA, indicating it had arisen as a *de novo* event in the affected individual (II-7).

Discussion

In dominant DEB, pathogenic mutations usually consist of glycine substitutions within the type VII collagen triple helix.[3–6] Glycine is the smallest amino acid and its presence at every third residue in the form of a Gly X–Y repeat is a characteristic feature of the coiled triple helix of several collagens, including type VII.[32,33] Substitution of glycine by a different amino acid disrupts the triple helix and leads to dominant-negative interference between wild-type and mutant protein. This may interfere with the secretion of type VII collagen and perturb anchoring fibril formation, thereby giving rise to fragility at the dermal–epidermal junction.[4,13]

Several different glycine substitution mutations in dominant DEB have now been described, and are highlighted in Fig. 7. Of these, only G2034R, G2043R and G2079R have been reported in more than one family. Most of the glycine substitutions that underlie dominant DEB lie within exon 73, the largest of the 118 exons that comprise COL7A1,[29] and most are clustered between amino acids 2003 and 2079.[3,5] Our study represents the fourth and fifth description of G2043R resulting in

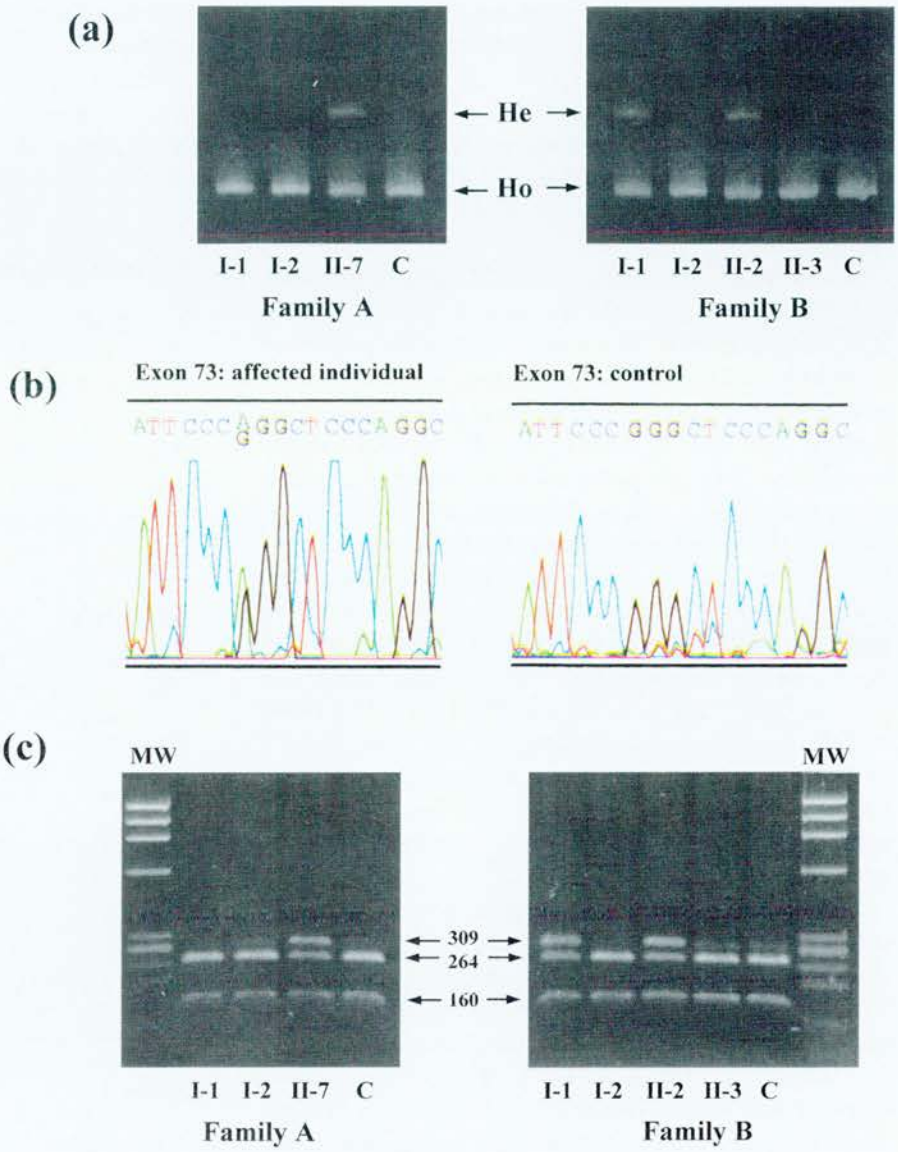

Figure 6. Identification of a shared COL7A1 mutation in families A and B. (a) Heteroduplex analysis of exons 73/74 in family A reveals a heteroduplex bandshift (He) in the polymerase chain reaction (PCR) product of the affected individual (II-7), but only homoduplex bands (Ho) in the amplified DNA from both parents (I-1 and I-2) and control (C). In family B, similar heteroduplex bandshifts are present in individuals I-1 and II-2, but not in I-2 or II-3 or the control sample. (b) Nucleotide sequencing of the PCR products spanning exons 73/74 in one of the affected individuals (here illustrated with DNA from individual II-2 in family B) reveals a heterozygous single G-to-A transition that converts a glycine residue (GGG) to arginine (AGG). The mutation is designated G2043R. (c) Verification of the mutation by restriction endonuclease digestion with *Sma*I. The control PCR products and those from the unaffected individuals in both families are digested into bands 264 and 160 bp in size. By contrast, the mutation results in loss of a *Sma*I cut site and an additional undigested band of 309 bp is seen in all the affected individuals (II-7 in family A, I-1 and II-2 in family B), indicating that they are all heterozygous for the mutation G2043R in exon 73 of COL7A1. MW, molecular weight marker.

this genodermatosis.[17,22,23] Thus, this mutation has now been found in Italian, Hungarian, Norwegian, Mexican and Scottish families. Furthermore, we are also aware of its occurrence in two other families with dominant DEB, one Finnish and one American (L.Pulkkinen, personal communication). Although a few recurrent mutations have been described in patients with autosomal recessive forms of DEB,[34–37] the mutation G2043R represents the first hotspot mutation to be elucidated in cases of dominant DEB. Interestingly, in families with this mutation, variability in the clinical features of the affected individuals is present. In the two families reported here, there was considerable interfamilial and intrafamilial heterogeneity. The Mexican

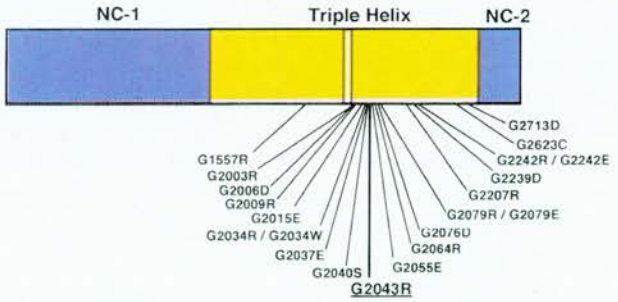

Figure 7. Database of published glycine substitution mutations in type VII collagen resulting in dominant dystrophic epidermolysis bullosa: G1557R;[14] G2003R;[15] G2006D;[16] G2009R;[17] G2015E;[16] G2034R;[16,18] G2034W;[19] G2037E;[20] G2040S;[21] G2043R;[17,22,23] G2055E;[14] G2064R;[19] G2076D;[18] G2079R;[18] G2079E;[24] G2207R;[18] G2239D;[25] G2242E;[25] G2242R;[26] G2623C;[27] G2713D.[19]

proband had features fully consistent with the Pasini form of dominant DEB, with numerous albopapuloid lesions on the trunk.[38] By contrast, in the Scottish family, individual II-2 (see family B, Fig. 2), of similar age to the Mexican patient, had less pronounced blisters and scarring, while his daughter (III-1) had less dystrophy but more extensive acral blister formation and inflammation.

Demonstration of the mutation G2043R in the Mexican family is only the third case of *de novo* dominant DEB to be reported. The rarity of *de novo* dominant mutations in COL7A1 has important implications for genetic counselling. Extensive mutation analysis in patients with mild DEB and clinically normal parents has shown that the overwhelming majority of these cases represents recessive DEB with a risk of affected children in subsequent pregnancies being one in four. By contrast, in cases of *de novo* dominant disease, the risk of having additional affected children is more difficult to predict because the recurrence risk arising from gonadal mosaicism is theoretically anywhere between that of the general population and 50%. However, in the Mexican family, none of the proband's 13 siblings was affected. It should be remembered, however, that for the affected individual in this family there will be a one in two possibility of her own offspring inheriting dominant DEB.

Although glycine substitution mutations in the triple helical domain of type VII collagen underlie most cases of dominant DEB, not all glycine substitutions actually lead to dominant-negative interference between mutant and wild-type protein. In particular, several silent glycine substitution mutations have been described,[3,5] and in individuals heterozygous for a COL7A1 allele bearing one of these mutations and a wild-type allele, there is no abnormal phenotype. However, if such a missense mutation is inherited on both alleles or in *trans* with a premature termination codon or splice site mutation on the other allele, the clinical consequences are either mild, moderate or severe recessive DEB, depending on the nature of the second mutation.[39–44] Precisely what determines whether a particular glycine substitution will lead to dominant-negative disruption, or alternatively function as a null allele, remains to be fully determined. In contrast to the silent glycine mutations, most of the substitutions that result in dominant DEB are located close to a non-collagenous interruption within the triple helix that may act as a hinge region, providing flexibility to the collagen. Disruption of this part of the protein may therefore have important implications for the functional integrity of type VII collagen.

In summary, we have detected a recurrent glycine substitution mutation in COL7A1. Elucidation of this mutation has significant implications for understanding the pathogenesis of skin fragility in dominant DEB and for the design of mutation detection strategies to investigate other patients with this mechanobullous disorder.

Acknowledgments

Support for this work was generously provided by Action Research, the Dystrophic Epidermolysis Bullosa Research Association (DEBRA, U.K.) and the St Thomas' Hospital Special Trustees. J.E.M. is the recipient of a DEBRA research fellowship. The co-operation of the families reported in this study and Fundación DEBRA México is gratefully acknowledged.

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Pyloric atresia–junctional epidermolysis bullosa syndrome: mutations in the integrin β4 gene (ITGB4) in two unrelated patients with mild disease

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Accepted for publication 2 June 1998

Summary Junctional epidermolysis bullosa associated with pyloric atresia (EB–PA; OMIM 226730) is a rare autosomal recessively inherited disease in which mucocutaneous fragility is associated with gastrointestinal atresia. This disease is usually fatal within the first few weeks or months of life even following surgical correction of the intestinal obstruction. Recently, mutations in the genes encoding the epithelial integrin α6β4 (ITGA6 and ITGB4) have been identified in several patients with EB–PA. We report two unrelated patients with this disease who have survived into early childhood with mild cutaneous involvement, in whom we have identified pathogenetic mutations in ITGB4. The first patient was a compound heterozygote for a splice site mutation in exon 30 (3793 + 1G-to-A) and a non-sense mutation in exon 36 (W1478X), and the second was a compound heterozygote for a missense mutation in exon 3 (C38R) and a 1 bp deletion in exon 36 (4776delG). Although the non-sense and deletion mutations are predicted to result in markedly reduced β4 integrin mRNA levels, the presence of the missense or splice site mutation on the second allele may enable the synthesis of some functional, albeit perturbed, β4 polypeptide. Determination of the molecular mechanisms in these two cases increases our understanding of EB–PA and may enable correlation between genotype and phenotype.

Junctional epidermolysis bullosa (JEB) is a group of autosomal recessively inherited mechanobullous diseases characterized ultrastructurally by a plane of blister formation through the lamina lucida of the basement membrane zone (BMZ).[1,2] Defective expression of proteins comprising the hemidesmosome–anchoring filament complex at the cutaneous BMZ, including laminin-5 and the 180 kDa bullous pemphigoid (BP) antigen, have been demonstrated in some forms of JEB and mutations in the corresponding genes identified.[3] Abnormal expression of integrin α6β4, also a component of hemidesmosomes in stratified and transitional epithelia, has been identified in a particular form of JEB associated with pyloric atresia (EB–PA) (OMIM 226730).[4–10] Other features of this disease include urogenital tract involvement, aplasia cutis and failure to thrive, with affected infants usually dying within the first few months of life.[11–15] Recently, mutations in the genes encoding the α6 and β4 integrin subunits (ITGA6 and ITGB4, respectively) have been described in EB–PA.[16–22] In this report, we describe two unrelated children with EB–PA, aged 6 and 3 years, with unusually mild cutaneous features of disease, although the older patient had marked urogenital tract involvement. Both had reduced expression of integrin α6β4 in biopsies of uninvolved skin. Molecular analysis of ITGB4 in the older child revealed compound heterozygosity for a splice site mutation and a non-sense mutation, while the younger child was a compound heterozygote for a single base deletion and a missense mutation. It is possible that these previously unreported combinations of mutations may be important in determining the relatively mild phenotype observed in these two patients.

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Materials and methods

Clinical details

Patient A. Some clinicopathological features of this case have been reported previously.[5,6] A male infant, currently 6 years old, was the first child of non-consanguineous Caucasian British parents. The proband was delivered normally at 35 weeks gestation following a pregnancy complicated by polyhydramnios. In the first days after birth, he was noted to have scanty blisters and erosions on his limbs, and dystrophic nails. He also had pyloric atresia and had a corrective gastroduodenostomy at the age of 6 days, from which he recovered without complication. In the third year of life, he developed haematuria and dysuria. Subsequent investigations revealed the presence of bladder wall haemorrhage and blistering, bilateral ureteric reflux, unstable detrusor contraction and recurrent urinary tract infection. He required frequent urological intervention and had a vesicostomy formed at age 5 years. In general, his skin remained in good condition with occasional small blisters and no mucous membrane involvement (Fig. 1a). His nail dystrophy persisted and he also had abnormal dentition with enamel hypoplasia. Both parents and a younger sibling are healthy.

Patient B. A male infant, now aged 3 years, was the only child of healthy Caucasian British parents who are not known to be related. He was delivered by forceps at 36 weeks gestation after a pregnancy complicated by polyhydramnios, gestational diabetes and maternal hypertension. Fetal hydronephrosis was detected antenatally. Pyloric atresia was diagnosed in the first few days after birth and at 6 days was corrected by pyloroplasty from which he recovered well. He had mild skin fragility to mechanical trauma and dystrophic nails which persisted (Fig. 1b). Postnatal ultrasound scans revealed no renal tract anomaly.

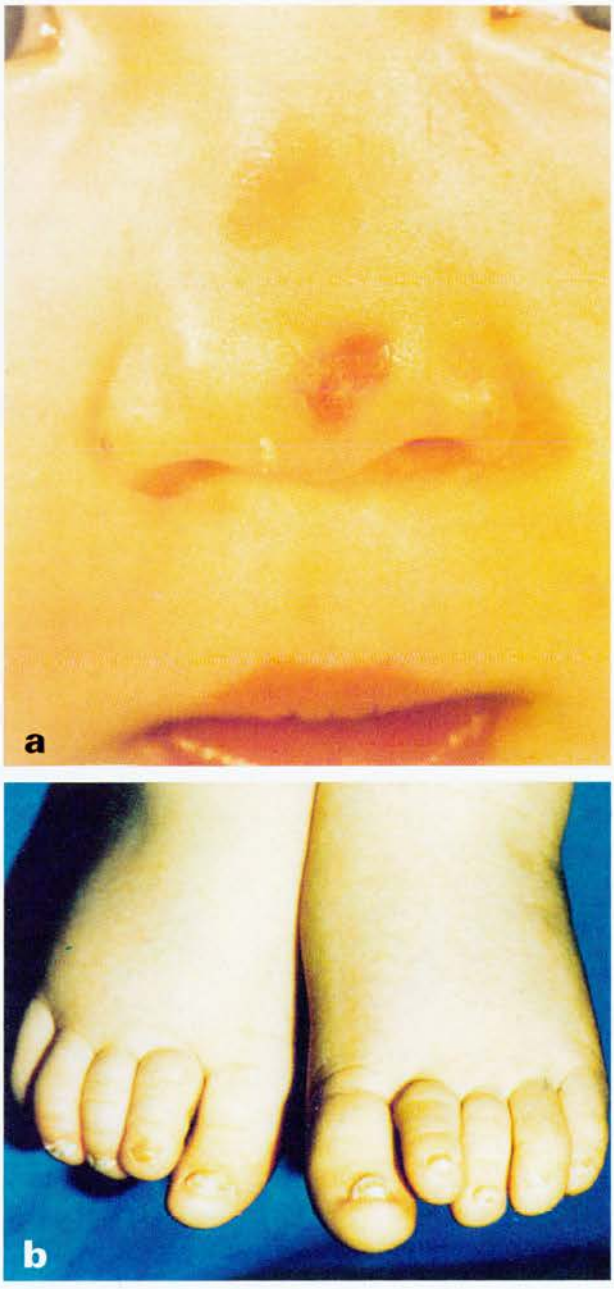

Figure 1. Clinical features of probands. (a) Small trauma-induced blister on the nose of patient A. (b) Toenail dystrophy in patient B.

Electron microscopy and immunohistochemistry

Biopsies of gently rubbed, uninvolved skin were taken from both probands under local anaesthesia. Samples for transmission electron microscopy were processed as described previously,[23] including primary fixation in half-strength Karnovsky fixative (containing 2% formaldehyde and 2·5% glutaraldehyde in 0·04 mol/L cacodylate buffer), and secondary fixation in 1·3% osmium tetroxide in distilled water. Samples were dehydrated in ethanol and embedded in Epon resin or Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEOL 100CX transmission electron microscope. Skin samples from patients A and B were processed for immunohistochemistry as described previously.[24,25] Cryostat sections were incubated with the following optimally diluted monoclonal antibodies or antisera: GB3 antilaminin-5 antibody[26] (Sera-Lab, Crawley Down, Sussex, U.K.); GoH3 antibody against the α6 integrin subunit[27] (gift from

A.Sonnenberg); 3E1 antibody (Gibco Life Technologies, Paisley, U.K.), G71 antibody[28] (gift from J.Aplin; patient A only) and 450–11A antibody[29] (gift from S.Kennel; patient B only) all against the β4 integrin subunit; 7A8 antiplectin antibody[30] (Sigma-Aldrich, Poole, Dorset, U.K.; patient B only); HD4–233 antibody against the 180 kDa BP antigen[31] (gift from K.Owaribe; patient B only); and FP-1 antiserum against the 230 kDa BP antigen[32] (gift from J.Stanley; patient B only).

Molecular analysis and verification of mutations

Genomic DNA from both probands, their parents and the unaffected sibling of patient A, was extracted from peripheral blood lymphocytes using a standard method.[33] Individual exons of ITGB4 (GenBank U66529-U66541) were amplified by polymerase chain reaction (PCR) using approximately 200 ng of genomic DNA as a template in standard conditions including 12 pmol of each primer, 200 μmol of dNTPs, 4% dimethyl sulphoxide, 10× PCR buffer and 1·25 U of AmpliTaq (Perkin Elmer Cetus, Norwalk, CT, U.S.A.) or Expand high-fidelity amplification reagents (Boehringer Mannheim, Indianapolis, IN, U.S.A.) in a 50 μL reaction volume. Specifically (see results), exons 3, 7, 30, 34 and 36 were amplified using the following pairs of primers based on flanking intronic sequences: for exon 3, sense, 5′-GCTGAG CACCTCCCCATTCA-3′; anti-sense, 5′-ATTCCCGACCCTTG CCTGTT-3′; for exon 7, sense, 5′-CCTGTGACACTCTCTC TCCC-3′; anti-sense, 5′-AGATTCCAGAACGCTAAAGC-3′; for exon 30, sense, 5′-CCTCGCCATGTCTGTCCATT-3′; anti-sense, 5′-TCCAGGGAGTGACCAAGAGC-3′; for exon 34, sense, 5′-GTGACCAGGAATGTGCAGGG-3′; anti-sense, 5′-CCAGGAGCTGGAAGAGAGGA-3′; and for exon 36, sense, 5′-GGGGGCAGCACTGTGACTCC-3′; anti-sense, 5′-AGGGACTTGGGTGGGTTCCT-3′. PCR conditions were 94 °C for 5 min followed by 35 cycles of 94 °C for 45 s, annealing temperature for 45 s and 72 °C for 45 s. Annealing temperatures were 64 °C for exons 3 and 36, 62 °C for exon 7 and 60 °C for exons 30 and 34. PCR products were examined on 2% agarose gels and then screened by heteroduplex analysis using conformation-sensitive gel electrophoresis (CSGE).[34] Exons displaying bandshifts were subjected to direct nucleotide sequencing (ABI, Perkin Elmer).

The mutations W1478X and C38R altered restriction endonuclease cut sites which were used to confirm the presence of mutations in the probands and family members (see Results). Specifically, W1478X created a *Nhe*I restriction enzyme site in the exon 36 PCR product from patient A (normal allele 327 bp, mutant allele 173 bp and 154 bp fragments), which was examined on a 2% agarose gel. C38R abolished a *Bbv*I restriction site in patient B's exon 3 PCR product (normal allele 112 bp and <40 bp fragments, mutant allele 136 bp and <40 bp fragments), which was viewed on a 4% NuSieve agarose gel (FMC BioProducts, Rockland, ME, U.S.A.). Restriction endonucleases were used according to the manufacturer's instructions (New England Biolabs, Hitchin, Herts, U.K.). The splice site mutation in exon 30, 3793 + 1G-to-A, in patient A, and the exon 36 deletion mutation, 4776delG, in patient B, did not alter a restriction enzyme site and therefore direct nucleotide sequencing was used to demonstrate the presence or absence of these mutations in genomic DNA from the parents of both patients and the unaffected sibling of patient A (see Results).

Results

Ultrastructural and immunohistochemical analysis of skin specimens from patient A have been described previously.[5,6] Electron microscopy of uninvolved skin from patient A demonstrated dermal–epidermal separation along the lamina lucida, whereas in patient B, there were areas of separation with a very low plane of cleavage in the basal keratinocytes above the hemidesmosome plaques (Fig. 2a). Hemidesmosomes were sparse and poorly formed with loss of sub-basal dense plates (Fig. 2b).

Immunohistochemical analysis using GB3 antibody against laminin-5 in skin biopsies from both probands revealed normal bright linear staining along the dermal–epidermal junction relative to normal control human skin (Fig. 3a,b). Immunoreactivity to GoH3 antibody against the α6 integrin subunit was markedly attenuated in patient B, but of near normal intensity in patient A (Fig. 3c,d). In both probands, immunoreactivity with antibody 3E1 against the β4 integrin subunit was greatly reduced relative to normal control skin, as was reactivity with the anti-β4 integrin antibodies G71, in patient A, and 450–11A, in patient B (Fig. 3e,f). In patient B, immunoreactivity to antibodies against the 180 kDa and 230 kDa BP antigens and plectin was moderately reduced compared with normal skin (data not shown).

In patient A, PCR amplification of ITGB4 followed by CSGE revealed heteroduplex bands in the PCR products spanning exons 30 and 36 (Fig. 4). Subsequent direct nucleotide sequencing of exon 30 and flanking intronic sequences demonstrated the presence of a heterozygous G-to-A transition at the 5′ donor consensus sequence at

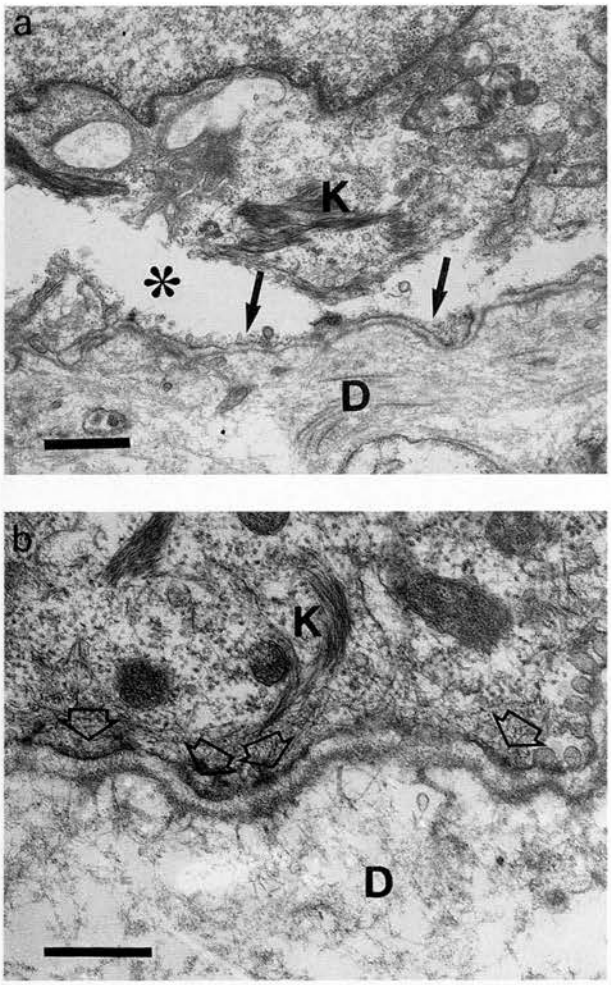

Figure 2. Electron microscopy of the dermal–epidermal junction in patient B. (a) Intracellular plane of cleavage (asterisk) with basal cell debris adherent to underlying basement membrane (arrows). Scale bar = 1 μm. (b) Poorly formed hemidesmosomes (arrows) with absent sub-basal dense plates (scale bar = 0·5 μm). K, keratin intermediate filaments; D, dermis.

the first nucleotide of intron 30 (3793 + 1G-to-A) (GenBank accession number U66529–U66541) (Fig. 4). Direct nucleotide sequencing of the exon 30 PCR product in the proband's parents confirmed that the mother was a carrier of this mutation. Sequencing of the exon 36 PCR product in patient A demonstrated a novel G-to-A transition at nucleotide position 4802, which converts a tryptophan residue (TGG) to a stop codon (TAG) (W1478X) (GenBank accession number X51841) (Fig. 4). This mutation creates a *Nhe*I restriction enzyme cut site and was used to confirm the presence of this mutation in the proband, his father and clinically unaffected sibling (Fig. 4), and to exclude it from 100 normal chromosomes.

Heteroduplex analysis of ITGB4 PCR products in patient B showed bandshifts in exons 3 and 36 (Fig. 5). Direct nucleotide sequencing of the PCR product spanning exon 3 revealed a novel heterozygous missense mutation arising from a T-to-C transition at nucleotide 112, which converts a cysteine residue (TGC) to an arginine (CGC) (C38R) (Fig. 5). This mutation abolishes a *Bbv*I restriction endonuclease cut site and therefore this enzyme was used to confirm the presence of C38R in the proband and his mother (Fig. 5), and to exclude it from 100 normal chromosomes. Sequencing of the proband's exon 36 PCR product demonstrated a heterozygous 1 bp deletion (4776delG), which results in a premature termination codon of translation 20 bp downstream (Fig. 5). Sequencing of this PCR product from parental DNA confirmed that the proband's father was a carrier for 4776delG. Heterozygous carrier status for this mutation was excluded from 100 normal chromosomes by sequencing or heteroduplex analysis.

Heteroduplex analysis of ITGB4 PCR products of the probands and family members also showed heteroduplex bands in some individuals in the PCR products spanning exons 7 and 34. Direct nucleotide sequencing of these PCR products disclosed two silent polymorphisms: 579T-to-C in exon 7 and 4521G-to-C in exon 34 (data not shown). The exon 7 polymorphism abolished a *Sty*I restriction site. Screening the exon 7 PCR product from 25 normal control subjects revealed that this polymorphism occurred on 17 alleles, giving an allelic frequency of 34% in this population. The polymorphism within exon 34 abolishes a *Bst*NI restriction site and was used to demonstrate this polymorphism on 29 alleles in the 25 control subjects, giving an allelic frequency of 58%.

Discussion

In this report, we have described two unrelated, British patients with EB–PA in whom pathogenetic mutations in the β4 integrin gene were identified. Both probands were unusual in that following surgical correction of pyloric atresia, they had survived into early childhood with minimal cutaneous involvement whereas the majority of patients with this disease die within the first few weeks or months of life from sepsis or other complications of the disease.

Integrin α6β4 is predominantly expressed in stratified squamous and transitional epithelia, and within the cutaneous BMZ is localized to hemidesmosomes which rely on the presence of this integrin for their

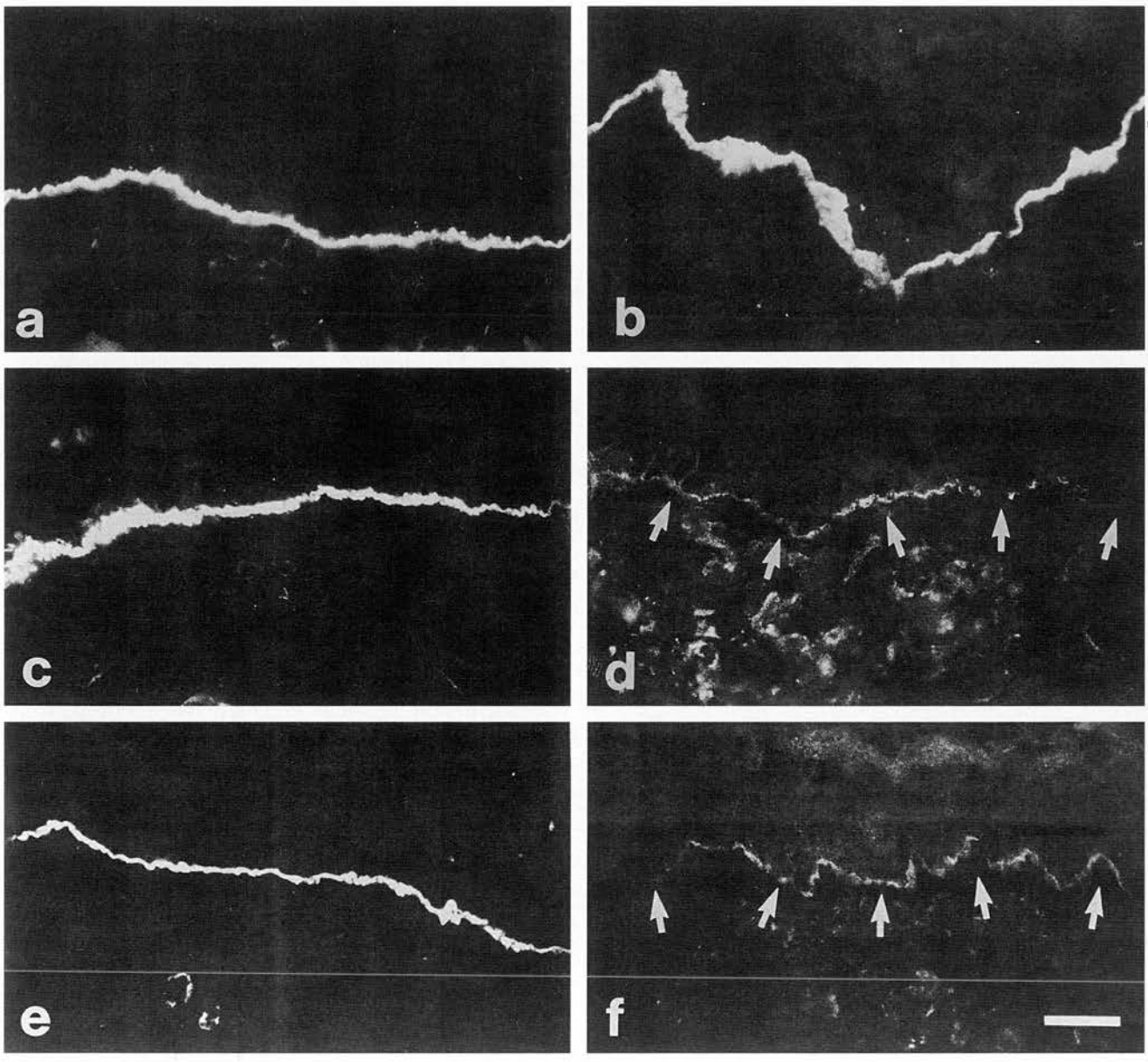

Figure 3. Indirect immunofluorescence of normal human control skin (a, c and e), and skin from patient B (b, d and f). Bright linear dermal-epidermal junction staining with GB3 antilaminin-5 antibody in both normal skin (a) and uninvolved skin from patient B (b). The dermal–epidermal junction in normal skin shows bright staining with GoH3 antibody against the α6 integrin subunit, and 450–11A against the β4 integrin subunit (c and e, respectively). Immunoreactivity with GoH3 and 450–11A is markedly reduced in uninvolved skin from patient B (d and f, respectively) (arrows) (scale bar = 25 μm).

formation.[8,35–39] The α6β4 heterodimer is involved in maintaining basal cell–matrix adhesion through interaction of the large intracytoplasmic tail of the β4 subunit with the keratin intermediate filament network, at least in part through its binding with plectin and the 180 kDa BP antigen.[40–42] Also, the extracellular domain of α6β4 integrin serves as a receptor for laminin-5 and interacts with the 180 kDa BP antigen, both of them being components of the lamina lucida.[43–45] The importance of integrin α6β4 in maintaining BMZ integrity has been demonstrated by mouse knock-out studies which resulted in dermal–epidermal separation with absent hemidesmosomes, associated with severe mucocutaneous fragility and gastrointestinal and urogenital tract abnormalities.[38,46,47]

The mutations identified in patients A and B, and

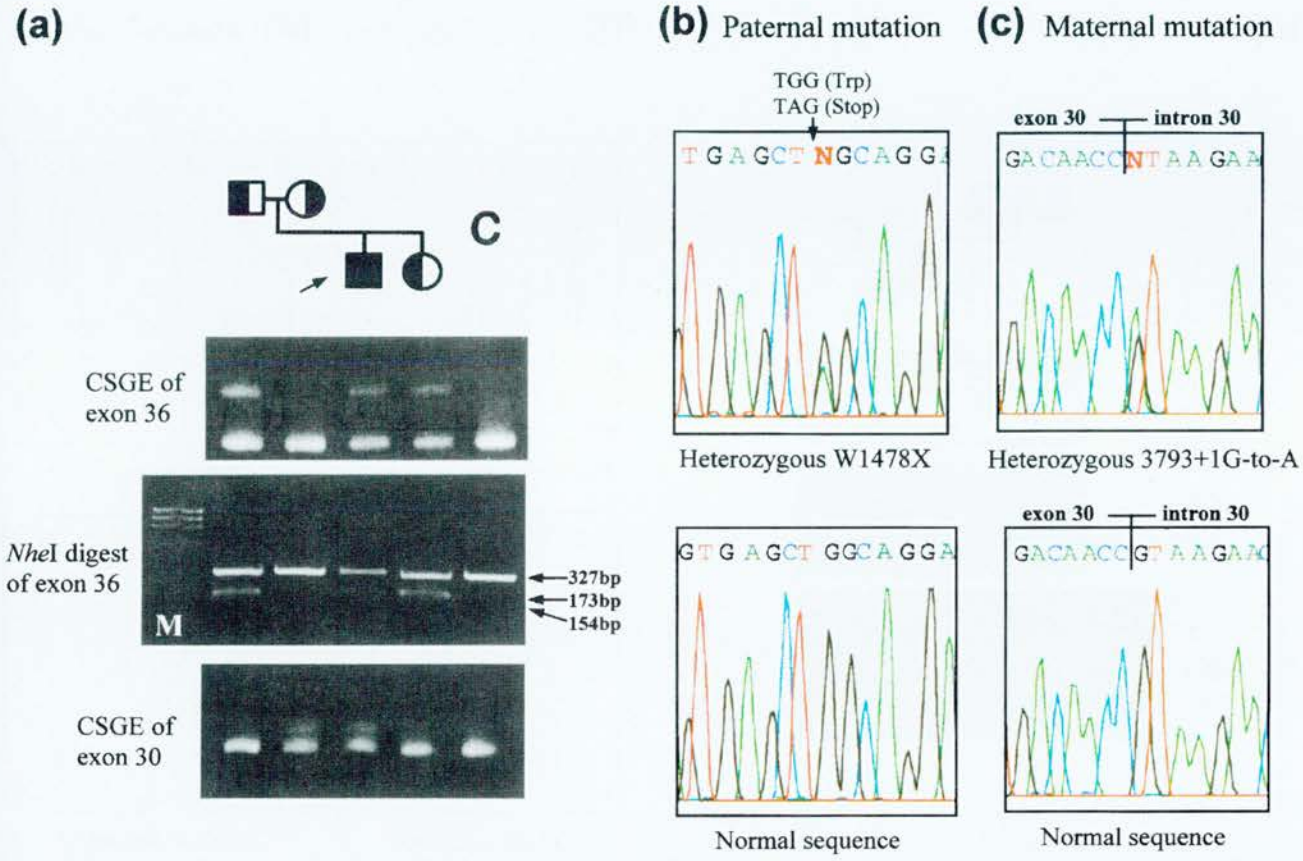

Figure 4. Mutation analysis in the family of patient A. (a) Conformation-sensitive gel electrophoresis (CSGE) analysis of polymerase chain reaction (PCR) products spanning exon 36 reveals heteroduplex bands in DNA from the father, proband and clinically unaffected sister, whereas the mother's and control DNA shows homoduplex bands only. The mutation W1478X creates a *Nhe*I restriction enzyme site which digests the mutant allele to 173 bp and 154 bp fragments, while the 327 bp normal fragment resists digestion. Restriction analysis with *Nhe*I of the exon 36 PCR products demonstrates that the father, proband and sibling are heterozygous for W1478X. CSGE analysis of exon 30 PCR products in this family demonstrates heteroduplex bands in the mother and proband, and homoduplex bands in the father, sibling and control DNA. (b) Direct nucleotide sequencing of the exon 36 PCR products reveals heterozygosity for a G-to-A transition at nucleotide position 4802, converting a tryptophan residue (TGG) to a stop codon (TAG) (W1478X) in patient A, his father, and unaffected sibling. Nucleotide sequencing of his mother's exon 36 PCR product is normal. (c) Direct nucleotide sequencing of exon 30 PCR products demonstrates a heterozygous G-to-A transition at the 5′ donor consensus sequence at the first nucleotide of intron 30 (3793 + 1G-to-A) in patient A and his mother, and normal sequence in his father and sibling. M, ϕX174 *Hae*III molecular weight marker.

previously published ITGB4 mutations in EB–PA, are shown diagrammatically in Figure 6. In our study, we found that patient A was a compound heterozygote for a splice site mutation (3793 + 1G-to-A) and a non-sense mutation (W1478X) in ITGB4, inherited on the maternal and paternal alleles, respectively. The splice site mutation has been demonstrated previously in a homozygous patient with EB–PA in whom mRNA studies suggested that two splice variants arose from the use of two cryptic splice sites which result in frameshifts and downstream premature termination codons and a markedly reduced mRNA level.[17] Compound heterozygosity for 3793 + 1G-to-A and the novel stop codon mutation, W1478X, in patient A might be expected to result in the synthesis of β4 polypeptides truncated in the intracytoplasmic tail domain, although non-sense-mediated mRNA decay is likely to result from these mutations and thereby the synthesis of little if any β4 integrin subunit.[48–50] Despite this, patient A has a milder phenotype than observed in another EB–PA patient homozygous for the same splice site mutation, as well as in most previously reported cases with ITGB4 mutations identified.[16–18,20,21] The reasons for this remain unclear, although mRNA studies of the proband might reveal the use of other, less disruptive cryptic splice sites arising from the 3793 + 1G-to-A mutation. If the W1478X allele is expressed at the protein level, the truncated polypeptide is expected to retain the

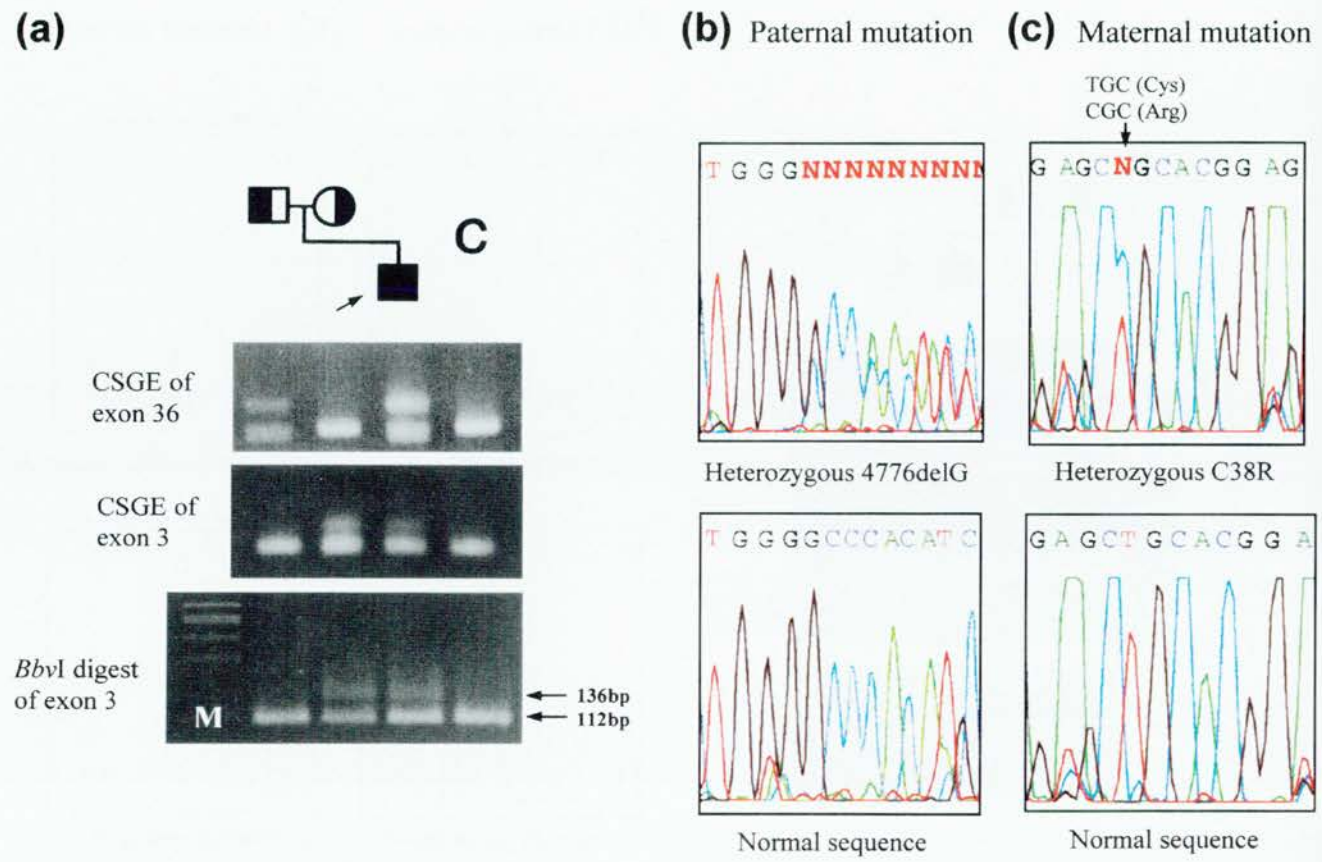

Figure 5. Mutation analysis in the family of patient B. (a) Conformation-sensitive gel electrophoresis (CSGE) analysis of polymerase chain reaction (PCR) products spanning exon 36 reveals heteroduplex bands in the proband and his father whereas PCR products from the mother and control show homoduplex bands only. Exon 3 PCR products demonstrate heteroduplex bands on CSGE analysis in the proband and his mother, but homoduplexes in his father's and control DNA. The mutation C38R in exon 3 abolishes a *Bbv*I restriction endonuclease site, digesting the normal allele into 112 bp and <40 bp fragments while digesting the mutant allele into 136 bp and <40 bp fragments. *Bbv*I analysis demonstrates the presence of C38R on one ITGB4 allele in the proband and his mother. (b) Direct nucleotide sequencing of exon 36 PCR products in this family reveals a heterozygous 1 bp deletion (4776delG) in the proband and his father. Maternal sequence of this exon is normal. (c) Sequencing of the proband's and mother's exon 3 PCR products demonstrates a heterozygous missense mutation arising from a T-to-C transition at nucleotide 112, converting a cysteine residue (TGC) to an arginine (CGC) (C38R). This mutation is not detected in the paternal PCR product. M, ϕX174 *Hae*III molecular weight marker.

binding site for plectin, while the binding site for the cytoplasmic domain of the 108 kDa BP antigen is deleted.[41,42]

Mutational analysis in patient B revealed compound heterozygosity for two novel mutations, 4776delG and C38R, on the paternal and maternal alleles, respectively. 4776delG results in a frameshift and downstream premature termination codon, also predictive of an unstable mRNA transcript from this allele. The missense mutation, C38R, arises in an exon of ITGB4 encoding part of the extracellular amino-terminal domain of the β4 integrin subunit. This amino acid substitution occurs in a region of the protein which is highly conserved between different β integrin polypeptides and between different species, and therefore might be expected to disrupt heterodimer formation with the α6 integrin subunit or interactions with ligands within the lamina lucida.[51–53] Perturbation rather than abolition of β4 subunit function by C38R may explain the mild phenotype of this proband with only minimal cutaneous involvement and no evidence of other sequelae. Recently, compound heterozygosity for a missense and a non-sense or premature termination codon mutation has been described in both lethal[21] and non-lethal[22] EB–PA, suggesting that both the position and nature of the amino acid substitution may influence phenotypic severity.

Ultrastructural analysis of uninvolved, gently rubbed skin from patient A revealed an intralamina lucida plane of cleavage. However, in patient B, the separation was intracellular and at, or just above, the level of the

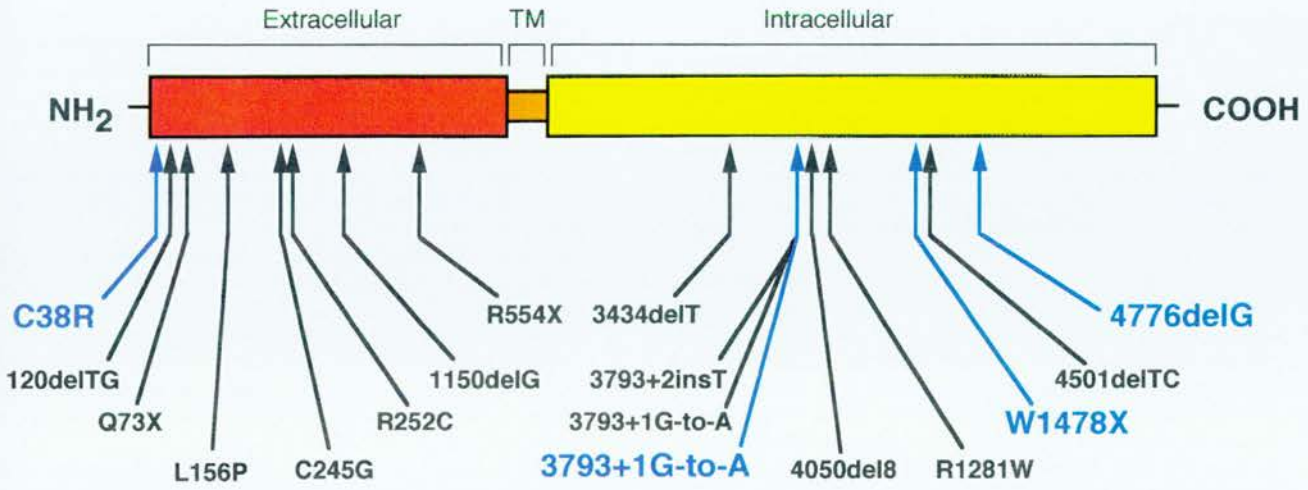

Figure 6. Database of mutations in ITGB4. Mutations described in this study are shown in blue. 1150delG, 3793 + 2insT;[16] 3793 + 1G-to-A;[17] 3434delT, 4050del8;[20] 4501delTC, 120delTG, C245G, Q73X;[21] L156P, R554X.[22] TM, transmembrane domain.

hemidesmosome plaques. Most cases of EB–PA reported have had a level of separation along the lamina lucida;[6,7,12–14,16,17,19–21,54] however, splits within the basal cell, and even below the lamina densa, have been reported in some patients,[15,21,55,56] as well as in knock-out mice with ablation of ITGB4 or ITGA6.[46,47] As integrin α6β4 comprises intracellular, transmembrane and extracellular domains, it is possible that mutations in ITGA6 or ITGB4 might give rise to a junctional or intracellular plane of cleavage at the BMZ. Further, the normal stable connections between the keratin filament network and hemidesmosomes are reduced in the skin of patients with EB–PA,[57] possibly rendering this peripheral cytoplasmic zone liable to rupture after trauma.

Immunohistochemical studies on skin from both patients demonstrated reduced immunoreactivity to antibodies against the β4 integrin subunit, and reduced immunoreactivity to the α6 subunit in patient B. The reduced α6 subunit expression in patient B relative to patient A may result from the C38R missense mutation which, causing an amino acid substitution in the extracellular domain of the β4 subunit, may greatly disrupt α6β4 heterodimer formation.[40] Similar findings with reduced expression of both integrin subunits have been demonstrated by other groups and underscore the close association with which this heterodimer is expressed at the basal epithelial cell surface.[7,9,16,21] Perturbed hemidesmosome assembly resulting from pathogenetic ITGB4 mutations is likely to account for the reduced immunoreactivity to the hemidesmosomal proteins plectin and the 180 kDa and 230 kDa BP antigens observed in patient B.

In addition, mutational analysis was undertaken to establish the frequency of two ITGB4 intragenic polymorphisms in a Caucasian European population. Both polymorphisms occur with a high allelic frequency (34% and 58%), which means that they may be useful markers for linkage studies, including DNA-based prenatal diagnosis, in other families with EB–PA.

In summary, we report two cases of mild EB–PA with mutations in ITGB4. Identification of these mutations increases our understanding of pathogenetic mechanisms in this disease and underscores the role of α6β4 integrin in maintaining epithelial–matrix adherence. Precise molecular classification in both affected families also has direct clinical relevance in that first trimester DNA-based prenatal diagnosis can be made available in subsequent at-risk pregnancies if requested.

Acknowledgments

The assistance of Dr Hans-Jürg Alder in providing DNA sequencing services at the Kimmel Cancer Institute, Jefferson Medical College, Jacqueline Denyer for assistance with clinical data, and Patricia Dopping-Hepenstal for technical assistance with electron microscopy at St John's Institute of Dermatology is gratefully acknowledged. The authors thank J.Aplin, S.Kennel, K.Owaribe, A.Sonnenberg and J.Stanley for generously providing antibodies. This work was supported by the Dystrophic Epidermolysis Bullosa Research Association (DEBRA, U.K.), a Wellcome Trust Research Travel Grant to J.E.M., and by USPHS, NIH grant PO1-AR38923.

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Laryngeal involvement in the Dowling–Meara variant of epidermolysis bullosa simplex with keratin mutations of severely disruptive potential

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Accepted for publication 1 October 1999

Summary The clinical features of the Dowling–Meara variant of epidermolysis bullosa simplex (EBS-DM) can, in an infant, be indistinguishable from other severe forms of epidermolysis bullosa (EB). Two unrelated infants with no family history of skin disease are described who, within hours of birth, developed extensive blistering of skin and oral mucosae and who both subsequently developed hoarse cries. Despite this superficial resemblance to other forms of EB, electron microscopy revealed a basal cell rupture and keratin aggregates characteristic of EBS-DM in the skin of both infants and in the vocal cord epithelium of one. Molecular analysis confirmed the diagnosis by identification of mis-sense point mutations in basal cell keratin genes in both cases. One patient carries a point mutation in keratin 14 (converting arginine at position 125 to histidine) and the other has a novel point mutation in keratin 5 (converting serine at position 181 to proline). Hoarseness is not a well documented feature of EBS-DM and is usually associated with junctional EB. These two patients demonstrate that the presence of a hoarse cry in an infant affected by severe EB does not necessarily indicate a poor prognosis.

Key words: Dowling–Meara, epidermolysis bullosa simplex, hoarseness, junctional epidermolysis bullosa, keratin 14, keratin 5, laryngeal blisters

Epidermolysis bullosa simplex (EBS) is a member of a larger EB family of disease which includes junctional EB (JEB) and dystrophic EB (DEB), which can be distinguished from each other histologically by the level of cleavage through the epidermis and by the fact that defects in distinct genes are responsible for each subtype.

EBS-Dowling–Meara (EBS-DM) is the most severe form of the three major EBS variants (DM, Koebner and Weber–Cockayne). The mutations that are responsible for the DM phenotype of EBS are at the ends of the helical rod domains of keratins 5 and 14[1] or more rarely with mutations in the intermediate filament-associated protein plectin.[2–4] These regions at the beginning and the end of the keratin rod domain (helix initiation and termination motifs) are highly conserved and have been shown to be critical for filament formation.[5–11] The severity of EBS is apparently dependent upon the nature of the mutation, its location in the protein and its effect upon keratin filament formation.

In the neonatal period, when there is often extensive blistering of the skin and mucous membranes, EBS-DM is clinically indistinguishable from both JEB and severe recessive DEB, which are associated with mutations in laminin 5,[12–15] bullous pemphigoid antigen-2[16] and type VII collagen,[17] respectively. By 12 months of age,

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clinical features characteristic of EBS-DM begin to emerge.[18] In common with all severe forms of EB, EBS-DM may be fatal during early infancy due to overwhelming sepsis but if this early stage is survived, life expectancy is normal. This is in contrast with the Herlitz type of JEB in which survival beyond 5 years is rare. A hoarse cry in a blistering infant has until now been considered indicative of JEB with a poor prognosis.[19–23]

We report two neonates with extensive blistering and hoarse cries who were shown by molecular and ultrastructure analysis to be suffering from EBS-DM. Mutations were found in keratin 14 in one patient and keratin 5 in the other. The mutation in the helix initiation motif of keratin 5 is the first reported at this site for a DM patient, and these are the first keratin mutations associated with laryngeal involvement.

Case reports

Patient 1

A male infant, born at term to non-consanguineous parents with no family history of skin disorders, developed widespread blistering of the skin and buccal mucous membranes within 24 h of birth. He was noted to have a persistently hoarse cry. A modestly raised maternal serum alpha-fetoprotein (2·05 multiples of the median) had been detected at 17 weeks of gestation, but a subsequent detailed ultrasound scan was reported as normal. Blistering remained widespread but became progressively more herpetiform (Fig. 1) with periungual involvement and nail dystrophy. The cry remained hoarse. At 7 months of age, direct laryngoscopy was undertaken and irregular thickenings were seen on both vocal cords.

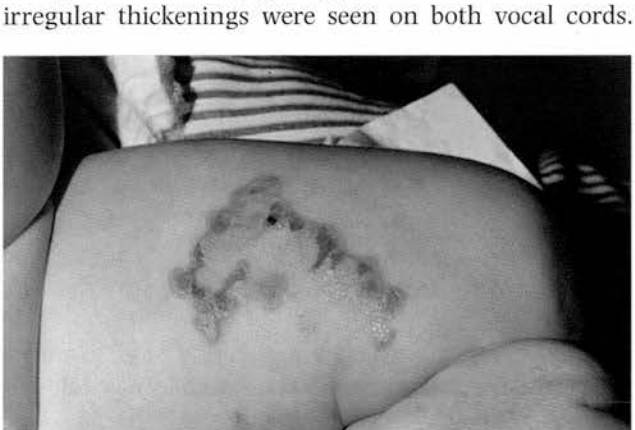


Figure 1. Herpetiform blistering with central clearing. Left thigh of patient 1 at 4 months of age, showing herpetiform blistering.

Hoarseness persisted until the age of 2 years. The child is now 6 years old and developing normally. Blisters remain widespread and herpetiform.

Patient 2

A female infant was born at term to healthy unrelated parents with no family history of skin disease. At birth there were blisters on the palms and soles and within hours blistering had spread to involve the tongue, buccal mucosa, periungual regions and large areas of skin. By 3 weeks of age she had developed stridor and a hoarse cry which was present until about the age of 2 years. Laryngoscopy was not undertaken. At the age of 19 months, the child developed a widespread cutaneous herpes simplex infection, requiring inpatient treatment with systemic aciclovir. She is now 5 years old and developing normally despite the persistence of widespread herpetiform blistering.

Methods

Electron microscopy

Fresh blisters from both infants, biopsied during the first week of life, and a biopsy taken at 7 months of age from the mucosa overlying the right vocal cord of patient 1, were processed for transmission electron microscopy.[24]

Mutation analysis

Genomic DNA was extracted from peripheral blood lymphocytes isolated from blood samples taken from both children, patients 1 and 2, and their parents for sequencing of the genes coding for keratins 5 and 14.

The keratin 14 genomic DNA for exon 1 was polymerase chain reaction (PCR) amplified using the sense primer 5′-TAC CCG AGC ACC TTC TCT TC-3′ (257–276 bp, EMBL accession number J00124), and the anti-sense primer 5′-TGC TGG AGA ACA AGT AGC TGC-3′ (1223–1202 bp). The cycle parameters for PCR amplification consisted of an incubation at 94 °C for 2 min followed by 30 cycles of 30 s at 94 °C, 1 min at 55 °C and 2 min at 72 °C, followed by 7 min at 72 °C. Approximately 500 ng of DNA was used per 100 μL reaction containing 60 mmol/L Tris–HCl pH 9·5, 15 mmol/L $(NH_4)_2SO_4$, and 2·5 mmol/L $MgCl_2$, 0·2 mmol/L each dNTP, 0·5 μg/mL each primer and 2·5 U *Taq* polymerase (Perkin Elmer Applied Biosystems, Warrington, U.K.). The PCR

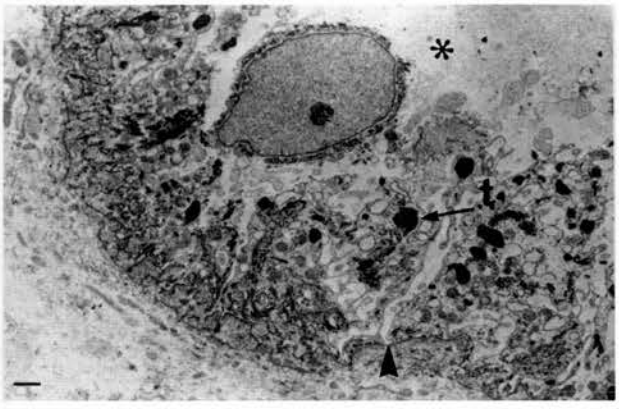


Figure 2. Electron microscopic analysis of a laryngeal biopsy from patient 1. Electron micrograph of a biopsy taken at 7 months of age from the right vocal cord, showing subnuclear cytolysis of basal keratinocytes, the lamina densa (arrowhead), blister cavity (asterisk) and clumped keratin tonofilaments (t). (Scale bar = 1 μm, original magnification × 3600.)

product of 967 bp was cloned directly into the pCR™II vector (Invitrogen, Abingdon, U.K.). The mutation was discovered in patient 1 in genomic DNA by dsDNA T7 sequencing (Pharmacia, Little Chalfont, U.K.) with [α-^{35}S]dATP (Amersham, Little Chalfont, U.K.), using the sense primer 5′-GGG GGA GCC TAT GGG TTG GGG-3′ (496–516 bp). The samples were resolved on 6% denaturing sequencing gels (National Diagnostics), and autoradiographed.

The keratin 5 genomic DNA (EMBL accession number M21389) for exon 1 was PCR amplified using the sense primer 5′-AAC AAG CCA CCA TGT CTC GCC AGT CAA GTG TGT CC-3′ (381–415 bp) and an intron 1 anti-sense primer 5′-CTC TTT GGC ATT TAT TTC AGA CCC-3′ under the same conditions as above. This fragment was used in sequencing and in the restriction analysis. The 623-bp fragment was cloned directly into the pCR™II vector (Invitrogen) and the mutation was detected, in patient 2, by cycle sequencing, using the above anti-sense primer end-labelled with [γ^{32}P]ATP (New England Biolabs, Hitchin, U.K.).

To prepare DNA fragments for restriction analysis, the PCR conditions were as above except for the addition of 0·7 ng of labelled anti-sense primer. One-tenth of the product was incubated at 60 °C for 15 min in a reaction consisting of 5 mmol/L spermidine, 100 μg/mL bovine serum albumin, 50 mmol/L NaCl, 10 mmol/L Tris–HCl, 10 mmol/L $MgCl_2$, 1 mmol/L dithiothreitol pH 7·9, and digested using 5 U of the restriction enzyme *Mnl*I and 5 U of *Ava*II at 37 °C overnight.

Results

Ultrastructural analysis

Ultrastructural examination of freshly blistered skin from both patients showed a cleavage plane through the subnuclear cytoplasm of basal keratinocytes, with tonofilament clumping and cytolysis (data not shown) characteristic of EBS-DM.[18] Ultrastructural examination of a biopsy taken from the mucosa overlying the right vocal cord of patient 1 showed features identical to those present in the skin (Fig. 2).

Identification of causative mutations

In most cases of EBS-DM, the mutations underlying the disease can be found in either keratin 5 or keratin 14, with most occurring in the helix initiation motif of keratin 14.[1] Using PCR, we amplified a fragment of the genomic keratin 14 DNA from patient 1 and subcloned it for sequencing. Sequencing of the individual alleles revealed a G to A substitution at the second nucleotide position in codon 125 of one allele of the keratin 14 gene, resulting in a predicted arginine to histidine substitution within the helix initiation motif of keratin 14 (K14R125H; Fig. 3). Parental DNA was unchanged at this position in keratin 14. This mutation is identical to several mutations already identified at this position in unrelated families with EBS-DM.[25,26]

When a keratin 14 mutation in patient 2 was not detected, genomic keratin 5 DNA was PCR amplified,

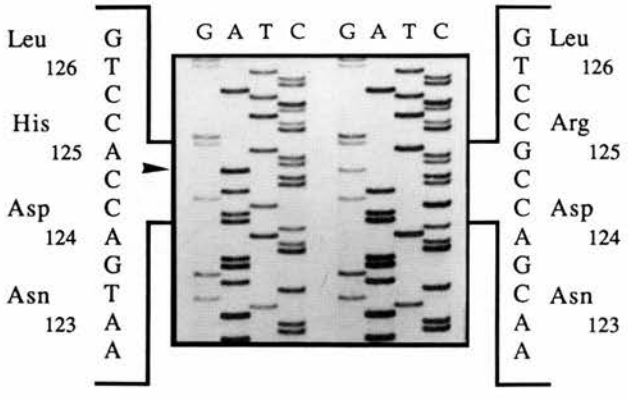


Figure 3. A mutation in keratin 14 encoding the α-helix 1A domain. Shown is the DNA sequence and predicted amino acid sequence of a portion of the region encoding the helix termination motif. The normal (right-hand side) and mutant allele (left) sequences from genomic DNA subclones. The G to A mutation (arrowhead) at codon 125 changes the predicted amino acid from arginine to histidine. Sequences were consistent in several clones. The asparagine codon shows a previously reported T to C silent heterozygous polymorphism.[45]

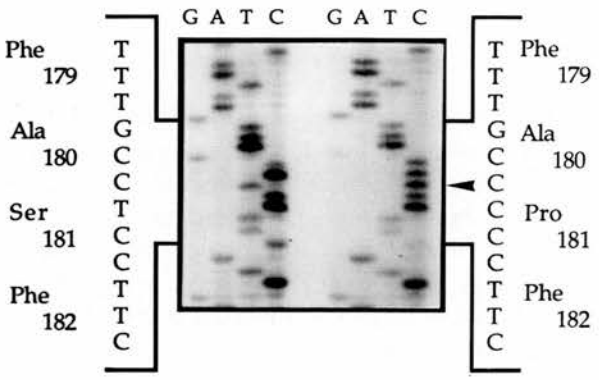


Figure 4. DNA sequence and predicted amino acid sequence of a portion of the region encoding the keratin 5 helix 1A domain. The DNA sequence shows the normal (left-hand side) and mutant allele (right-hand side) sequences from genomic DNA clones. The T to C mutation (arrowhead) at codon 181 changes the predicted coding amino acid from a serine to a proline. Sequences were consistent within several clones.

subcloned and sequenced. Figure 4 shows the sequence change identified in one allele of patient 2, as a T to C substitution at the second nucleotide position at codon 181. The substitution predicts a change of the amino acid encoded from a serine to a proline (K5S181P). This is the first report of a mutation at this position in keratin 5 for a DM patient. More than 50 non-EBS individuals, plus the parents, were screened and found negative for the mutation, indicating that it is not a common polymorphism in the population (Fig. 5).

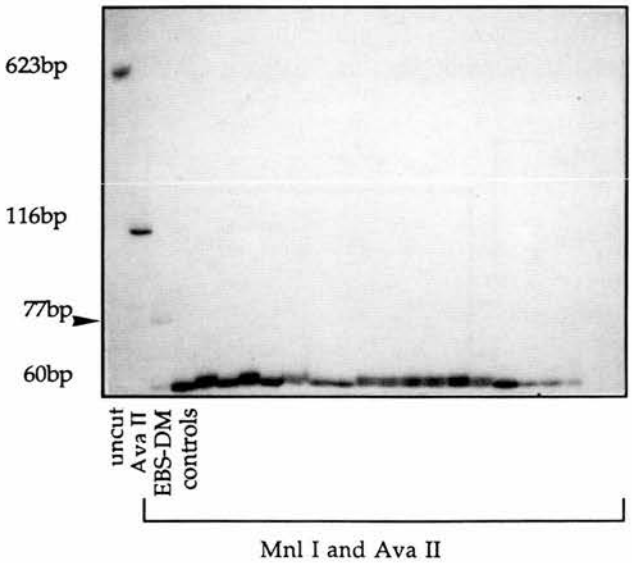


Figure 5. The restriction digest screen demonstrating that the keratin 5 (S181P) mutation is not present in the normal population. Digestion with *Ava*II of a 3′-end-labelled polymerase chain reaction product was used to remove numerous *Mnl*I sites present in the full-length fragment (623 bp). The resulting 116 bp labelled fragment was then cleaved with *Mnl*I to produce a 60-bp fragment in normal sequences, and a 77 bp fragment (arrowhead) in DNA carrying the T to C mutation which knocked out the *Mnl*I site.

Discussion

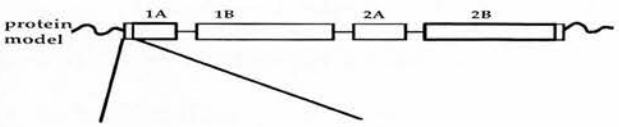
Both neonates presented, within hours of birth, with extensive blistering indistinguishable clinically from JEB and severe recessive DEB. The principal point of clinical interest in these two infants was their hoarseness. Hoarseness is well recognized in JEB in which laryngeal involvement may be an important cause of death.[27] This has not been a well documented feature of EBS-DM, although it has been noted in one previous report[28] and in two patients with plectin defects associated with EBS and muscular dystrophy.[29]

In our patients, identification of point mutations in keratins 5 and 14 as well as the typical ultrastructural defects were diagnostic, thus enabling an accurate assessment of the prognosis in each case. An incidental finding in patient 1 was the detection of a raised maternal serum alpha-fetoprotein on routine screening at 17 weeks of gestation. Although most often associated with open neural tube defects this has been described in association with severe fetal dermatoses[30–33] including EBS.[34]

The R125H mutation in the helix initiation motif of keratin 14 identified in patient 1 is one of the most common causes of EBS-DM, and this codon is the most commonly affected in all of the keratin disorders. This reflects not only a high mutability of this codon, as it contains a CpG dinucleotide, but it also reflects a great functional sensitivity of this region of the protein to any amino acid sequence variation.

In EBS-DM cases, mutations in keratin 5 have been reported less frequently to date than mutations in keratin 14. They include E475G,[35] E477K,[36] E477stop[37] and I466T[38] in the helix termination motif, and L174F,[39] N176S.[36,40] F179S[36] and a splice site mutation[41] in the helix initiation motif.

The serine at position 181 in keratin 5 is a highly conserved amino acid across keratins and other intermediate filament proteins (Fig. 6), indicating the important role of this residue. A similar mutation at this position has been reported in keratin 1 for a case of bullous congenital ichthyosiform erythroderma,[42] although it has not before been reported with EBS-DM. A proline residue is predicted to be very disruptive to the α-helix, and thus to keratin dimer or filament formation.[9,42] Here it is believed to have



IKTLNNKFA**S**FI	keratin 5
IKTLNNKFA**P**FI	EBS-DM S181P
IKSLNNQFA**S**FI	keratin 1
IKTLNNKFA**S**FI	keratin 2e
MQELNSRLA**S**YL	keratin 9
MQNLNDRLA**S**YL	keratin 10
MQNLNDRLA**S**YL	keratin 14
LQELNDRFA**N**YN	desmin - chicken
LQELNDRFA**N**YI	vimentin - human
MMELNDRFA**S**YI	GFAP
LQDLNDRFA**S**FI	NF-L
LQGLNDRFA**G**YI	NF-M
LQDLNDRFA**S**FI	snail IF
LSHLNDRFA**S**YI	BrIf-1
abcdefgabcde	heptad repeat

Figure 6. Conservation of the keratin 5 serine residue 181 across intermediate filaments. A schematic representation of type 1 and type 2 keratin intermediate filament proteins which shows the central rod domain and a comparison of a portion of the amino acid sequence of the helix initiation motif. The italic lettering refers to positions within the heptad repeat. Also shown are the amino acid sequences at the helix initiation motif of several other intermediate filament proteins including GFAP (glial fibrillary associated protein), NF-L (neurofilament light), NF-M (neurofilament moderate) and the cephalochordate *Branchiostoma lanceolatum* (BrIf-1).

contributed to severe EBS-DM (patient 2), with involvement of the larynx. The high degree of conservation of the serine residue, its position in the helix initiation motif, and the amino acid change from serine to proline predict that this would produce a severe phenotype.

By electron microscopy, sporadic electron dense aggregates of keratin were clearly seen in the basal keratinocytes of the stratified squamous epithelium of the vocal folds. Keratins 5 and 14 are the major keratin proteins of basal layer cells in this type of tissue, and the aggregates here are consistent with the observed mutations. Disruptive dominant negative mutations in these keratins give rise to keratin aggregates instead of correctly assembled filaments, and as in the epidermis, this leads to an inherent fragility in the affected cells, and subsequent cytolysis on trauma (Fig. 2) due to the lack of an effective intracellular supportive cytoskeleton. As vocal cords contain a stratified squamous epithelium, it is perhaps surprising that hoarseness and laryngeal blisters have been described so infrequently in EBS. The keratin 5 and 14 mutations reported here are potentially very disruptive to the keratin intermediate filament, which may, perhaps in combination with other genetic factors of the individual,[43,44] have resulted in the detection of rarely reported laryngeal involvement in EBS-DM.

These two neonates, with extensive blistering, nail involvement and hoarse cries, have features clinically indistinguishable from JEB, but have been shown by keratin gene sequence analysis, ultrastructural examination of the larynx of one and the skin of both, to be suffering from the EBS-DM.

Acknowledgments

Thanks to Dot Mechan and David Baty, Human Genetics Laboratory, Ninewells Hospital, Dundee for performing the genomic DNA extraction and Gordon Milne, Department of Histopathology at Ninewells Hospital for the electron microscopy. This work was supported by grants from DEBRA (the Dystrophic Epidermolysis Bullosa Research Association), and the Cancer Research Campaign (grant no. SP2060).

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British Journal of Dermatology 2000; **142:** 468–472.

The clinical spectrum of epidermolysis bullosa simplex

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Accepted for publication 28 October 1999

Summary As part of the U.K. National Epidermolysis Bullosa Register, we have systematically recorded clinical information on 130 (77%) of the 168 known Scottish epidermolysis bullosa simplex (EBS) sufferers. Three subtypes of EBS were recognized: Dowling–Meara (EBS-DM), Weber–Cockayne (EBS-WC) and Köbner (EBS-Kb), seen in 5%, 42% and 53% of patients, respectively. As there is considerable overlap between EBS-WC and EBS-Kb, with both phenotypes frequently seen within the same pedigree, EBS-WC is best regarded as a milder variant of EBS-Kb rather than a separate disorder. Improvement with age is common in all variants of EBS, but is not invariable. Pain due to acral blistering in EBS-Kb/EBS-WC has a more marked impact on life-style than the blisters of EBS-DM. Oral blistering, nail involvement and aplasia cutis congenita occur in all EBS subtypes and laryngeal involvement is a feature of EBS-DM. Seasonal variation is not seen in EBS-DM but is common in EBS-Kb/EBS-WC.

Key words: epidermolysis bullosa simplex, epidermolysis bullosa

Since 1992, as part of the U.K. National Epidermolysis Bullosa Register,[1] clinical details of all known Scottish epidermolysis bullosa (EB) patients have been systematically recorded. In view of a relative paucity of documentation of clinical features in the more common milder forms of EB, we have attempted to record the complete clinical spectrum of EB simplex (EBS) within the Scottish population.

EBS, characterized by a cleavage plane through basal keratinocytes, is caused by mutations of genes encoding keratins 5 and 14, and plectin.[2–7] It is often regarded as the least severe form of EB. Although usually not life threatening, EBS-Dowling–Meara (EBS-DM), the most severe subtype,[8] can, like both junctional and recessive dystrophic EB, be fatal during infancy.[9] In 1886, Köbner described seasonal blistering of predominantly the palms and soles, but occurring, in addition, at other sites of friction from clothing such as beneath sock garters, in the groin and under corsets.[10] His name is often used to describe EBS in which blisters are generalized,[9] but in this paper we use the term EBS-Köbner (EBS-Kb) to describe patients who fit Köbner's original description, and EBS-Weber–Cockayne (EBS-WC) to describe blistering confined exclusively to the palms and soles.[11,12]

Patients and methods

One hundred and sixty-eight individuals in Scotland are known to suffer from EBS. One hundred and forty-nine were identified and assessed during compilation of the EB Register,[1] and a further 19 were subsequently referred to the Department of Dermatology at the Royal Infirmary of Edinburgh. Detailed clinical information has been recorded on 130 patients from 33 families. Of the remaining 38 patients, 30 are affected relatives, identified by the probands, who have not yet been interviewed. Incomplete information exists on a further five unrelated patients. The remaining three patients have been diagnosed at other Scottish hospitals as suffering from EBS, but have not yet been seen by the authors. We report the clinical features seen in the group of 130 patients for whom information is complete.

Results

The three most common subtypes of EBS were encountered in this group of Scottish patients: EBS-DM (5%), EBS-WC (42%) and EBS-Kb (53%).

The Dowling–Meara subtype of epidermolysis bullosa simplex

This was the most severe variant of EBS and was identified in seven patients aged between 3 weeks and 49 years at initial interview. The two youngest were identical twins, but the remaining five patients were unrelated. None had an affected parent. The eldest (and only patient over 18 years of age) had an affected son, for whom no clinical information is available and who was therefore not included in the study. Blisters were congenital in four, and appeared during the first week after birth in the other three (Table 1). One of the seven was born with an area of absent epidermis on one leg. All experienced widespread blistering which, after the first few months of postnatal life, took on the clustered herpetiform appearance characteristic of EBS-DM.[13] The severity of blistering lessened during childhood and adolescence in all patients. The four infants in the study had intraoral blistering and hoarse cries. Two have been followed up for several years; their hoarseness resolved during their third year. All subjects had nail involvement. In the case of the infants, this consisted of periodic shedding of finger and toe nails which subsequently regrew. The adults had thickened great toe nails. None experienced any seasonal variation of the blistering tendency. Mild hyperkeratosis of the weight-bearing areas of the soles was seen in the two oldest patients, aged 17 and 49 years. Although friction from close-fitting clothing was an important cause of blisters in all patients with EBS-DM, many blisters appeared to arise spontaneously. Scarring was not seen in any subjects, although widespread post-inflammatory pigmentation was present in one of the two adolescents. This patient also had several atypical naevi, apparently at the sites of previous blisters.

Table 1. Age at onset of blistering in patients with epidermolysis bullosa simplex (EBS)

Age at onset	All patients	EBS-WC	EBS-Kb	EBS-DM
Birth	9	1	4	4
1–7 days	16	3	10	3
1–4 weeks	10	2	8	0
1–6 months	22	2	20	0
7 months–2 years	34	20	14	0
2–5 years	8	7	1	0
6–10 years	6	4	2	0
11–20 years	2	2	0	0
Adult	0	0	0	0
Unknown	23	13	10	0
Total	130	54	69	7

EBS-WC, Weber–Cockayne subtype of EBS; EBS-Kb, Köbner subtype of EBS. EBS-DM, Dowling–Meara subtype of EBS.

Ultrastructural examination of fresh blisters from one twin and four others showed, in each case, characteristic cytolysis of basal and suprabasal keratinocytes and clumping of keratin tonofilaments.[13] Identical changes were seen in a biopsy from the vocal cord of one infant.[14] The seventh and oldest patient, for whom no ultrastructural information is available, has been shown to carry a mutation of a keratin 14 allele identified previously in other EBS-DM sufferers (K14Leu^{122}Phe).[15] Analysis of keratin genes has been undertaken in the remaining patients and, in each case, a causative mutation has been identified (K5Ser^{181}Pro, a novel mutation not previously reported; Professor E.B.Lane, personal communication; K14Arg^{125}Cys;[16–20] and K14Arg^{125}His,[16,17,21] found in two patients).

The Köbner subtype of epidermolysis bullosa simplex

Sixty-nine individuals, while experiencing blisters predominantly on the palms and soles, also blistered at other sites. Seventeen families were represented. Four patients had parents who had never blistered. Patients were aged between 1·3 and 75·5 years at interview (mean 29·2, median 29·5). All experienced blisters on the feet and other sites. Sixty-three (91%) suffered blisters on the palms. Intraoral blisters occurred in 17 subjects (24%), and the great toe nails were thickened in 10 patients (14%) aged between 1·5 and 57·1 years. Mild hyperkeratosis of the weight-bearing areas of the soles was present in eight adults (12%), four of whom were members of one family. Sixty subjects (87%) indicated that their blistering tendency was worst in warm weather. Only 11 (16%) were completely free of blisters during the winter. Three patients (4%), two of whom were mildly affected, denied seasonal variation in severity of blistering. Six children (9%) were too young to allow assessment of seasonal variation.

Friction from clothing and jewellery was identified by patients as an important cause of blisters, with heat a contributory factor, sometimes apparently causing blisters even in the absence of friction. Improvement with age was noted by 20 patients (29%), only two of whom (aged 3 and 11 years) were under 20 years old. Fourteen patients (20%), aged 5–57 years, felt there had been no improvement in blistering as they had grown older. Of these, seven were over 20 years old. Eight children were too young to allow any assessment of improvement, and no information is available for the remaining 27 patients (39%) whose ages range from 4

to 67 years (mean 26·8). While the age at onset of blistering in EBS-Kb varied between birth and 10 years of age, 81% developed their first blisters before the age of 2 years (Table 1). Two patients, mother and daughter, were each born with a single absent area of epidermis on one leg, which subsequently healed leaving subtle atrophic scarring. In one family, all three affected children experienced blisters in the anogenital region.

There were six families, none exceeding four sufferers, in which all affected individuals had the EBS-Kb pattern of blistering. In each of the remaining 11 pedigrees, ranging in size from two to 25 affected individuals, there was a mixed pattern of blistering, with both EBS-WC and EBS-Kb phenotypes represented.

The Weber–Cockayne subtype of epidermolysis bullosa simplex

EBS-WC was seen in 54 patients from 20 families. Patients were aged between 1·3 and 78·3 years (mean 27·8, median 24·6) at interview. Six patients had unaffected parents. Blisters were confined to the hands and feet, although four individuals (7%) from three families also experienced intraoral blistering. Only two sufferers did not have sole involvement: one (aged 15·5 years) had mild involvement of the palms in early childhood and the other (aged 24 years) experienced palmar blisters which were exacerbated by his manual occupation. Palmar blistering was a feature in only 31 patients (57%). Thickened great toe nails were present in seven individuals (12%), three of whom were aged under 4 years. No fungus was found in any nail clippings submitted for mycological examination. Five patients (9%), all adults, had mild hyperkeratosis of the soles. Seasonal variation of blistering, worst during the summer, was noticed by all but four very mildly affected adults (93%). Twenty-seven individuals (50%) continued to experience blisters to a lesser extent during the winter, while 11 (20%) were completely free of blisters during the colder months. Three children were too young to allow assessment of seasonal variation, and information is incomplete for the remaining 13 (24%) patients.

As in EBS-Kb, blisters were attributed to friction and heat. In 19 patients (35%), the blistering tendency had improved with age, all but one being over 20 years old. Seventeen (31%) had not improved, although only three were over the age of 20 years. One child (aged 1·3 years) was too young to allow assessment of improvement, and no information was available for the remaining 17 patients (31%), aged 3·5–51 years. The age at onset of blistering was slightly later than that seen in EBS-Kb, varying between birth and the teenage years (Table 1). Fifty-three per cent had experienced their first blisters by the age of 2 years. One child was born with a localized area of absent epidermis on one leg.

There were nine pedigrees, none exceeding four patients, in which all affected individuals demonstrated exclusively the EBS-WC phenotype.

Effect of epidermolysis bullosa simplex on life-style

In both EBS-WC and EBS-Kb, foot blisters frequently resulted in pain sufficient to require transport even over short distances, and to limit walking during the summer months. At home, it was common for affected children and teenagers to avoid walking; they preferred to crawl or 'bottom shuffle'. One adult EBS-Kb patient used a wheelchair during the summer months. Some parents carried their affected children to primary school. Teenagers often found coping with frequent changes of classroom during their school day difficult, the less motivated regularly missing school because of pain due to blisters. Two families had persuaded their local education authorities to provide taxis to take their affected children to school. Fourteen sufferers (8%; nine with EBS-Kb, four with EBS-WC and one with EBS-DM) were receiving social security benefits which in 11 cases was a mobility allowance. Seven of 67 adults of employable age (10%) were unemployed, five of whom suffered from EBS-Kb and two from EBS-WC. Both adults affected by EBS-DM were in full-time employment.

Discussion

Comparison with other EB databases suggests that this population-based study is equally if not more rigorous than other national surveys.[1,22–27] We have tried to minimize any bias towards more severely affected patients by actively seeking out EBS sufferers, approximately one-third of whom proved to be previously unknown to dermatologists.[1] It is inevitable that some patients will remain undetected. Extrapolation and analysis of data for the Lothians, the catchment area of the Royal Infirmary of Edinburgh, suggests the national prevalence of EBS to be appreciably higher than recorded in any studies to date.[1]

Localized areas of congenital aplasia cutis, originally termed 'Bart's syndrome'[28] and since recorded in all

major EB subtypes,[29] are seen in all three variants of EBS. Early onset and intraoral blisters, features typically associated with recessive dystrophic and junctional EB,[9] all occur in each variant of EBS. Widespread blistering during infancy in those few affected by EBS-DM necessitates intensive nursing care, but over the longer term, pain has a greater and more prolonged effect on the life-style of those affected by EBS-Kb and EBS-WC.

EBS is a spectrum, being a potentially life-threatening condition for those few affected by EBS-DM, a painful disability for many and little more than an inconvenience for some. There is considerable overlap within this spectrum, to such an extent that EBS-WC and EBS-Kb should no longer be considered different disorders. Both phenotypes are seen in family members carrying identical causative mutations, and this is apparent in all Scottish families of five or more EBS sufferers. Thus, both EBS-Kb and EBS-WC appear to represent variations of the same condition. Similar as yet unexplained phenotypic variation is seen in other single gene disorders, e.g. cystic fibrosis, neurofibromatosis and ichthyosis bullosa of Siemens.[30–32] It appears the causative mutation, although important, is not the only factor determining clinical severity.

Acknowledgment

We are grateful to the Dystrophic Epidermolysis Bullosa Research Association for funding this work.

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British Journal of Dermatology 2002; **146:** 267–274.

Clinical Investigations

The clinical spectrum of dystrophic epidermolysis bullosa

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Accepted for publication 3 October 2001

Summary

Background Dystrophic epidermolysis bullosa (DEB) is a genodermatosis resulting from mutations in *COL7A1*, the gene encoding type VII collagen. The site and specific nature of the underlying mutation determine the clinical phenotype, which ranges widely from a severe mutilating condition to a relatively mild disorder.

Objectives To document the clinical spectrum of DEB within a defined complete population.

Methods Since 1992, when compilation of the U.K. epidermolysis bullosa register began, an exhaustive search for DEB sufferers within the Scottish population has been undertaken and their clinical features comprehensively recorded.

Results One hundred and twenty-eight DEB sufferers have been identified within the Scottish population. In descending order, the frequencies of the different forms of DEB were dominant DEB (DDEB) in 88 individuals (68%), DEB of uncertain inheritance in 24 (19%) and recessive DEB (RDEB) in 16 patients (13%). Within this latter group, nine (7%) had the mutilating Hallopeau–Siemens subtype (RDEB-HS), five (4%) had localized (RDEB-loc) and two (2%) had a predominantly flexural (inverse) form of RDEB. During the study, two patients with RDEB died from squamous cell carcinomas (SCCs), one originating in the skin and the second arising in the oesophagus. Gastrointestinal problems such as dysphagia, constipation and anal fissures, and restriction of mouth opening were experienced by the majority of patients with RDEB and by a significant minority of DDEB sufferers. Pseudosyndactyly was most severe in RDEB-HS, all those over 9 years of age having mitten deformities of the hands. Milder pseudosyndactyly or flexion contractures of the fingers were present in younger patients with this subtype, in most adults suffering from other subtypes of RDEB and in 6% of those with DDEB. External ear involvement, a feature not often reported in DEB, was common in RDEB and also occurred in a minority of those with DDEB. Pruriginous lesions and albopapuloid lesions were each present in both DDEB and RDEB.

Conclusions Most patients with DEB have relatively mild dominantly inherited disease, only a minority suffering from severe recessive subtypes. Scarring, gastrointestinal involvement, albopapuloid lesions and a pruriginosa-like pattern each occur in both DDEB and RDEB. With increasing age, SCC is a major cause of morbidity and mortality.

Key words: albopapuloid lesions, dystrophic epidermolysis bullosa, epidermolysis bullosa pruriginosa, epidermolysis bullosa register

Dystrophic epidermolysis bullosa (DEB) is a genodermatosis resulting from mutations in *COL7A1*, the gene encoding type VII collagen. The site and specific nature of the underlying mutation determine the clinical phenotype, which ranges widely from a severe mutilating condition to a relatively mild disorder.

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Because of its unique characteristics, Scotland was selected for compilation of the first section of the U.K. epidermolysis bullosa (EB) register. This part of Britain has the advantages of being both politically and geographically well defined; its population of 5 million[1] is small enough to permit thorough assessment while being large enough to reveal the full clinical spectrum of EB. Those affected by severe variants of EB are well

Table 1. Prevalences of epidermolysis bullosa (EB) (per million)

	EBS	JEB	DEB
Scotland[a]	32·0	0·4	24·4
Norway[2]	24·3	–	9·3
N. Ireland[3]	28·0	0·7	3·3
Finland[4]	15·1	0·2	8·8
Croatia[5]	1·5	1·5	6·6
Japan[6]	4·0	0·2	3·5
Saudi Arabia[7]	1·7	0·0	3·7
S. Africa[8]	0·8	0·7	1·2
U.S.A.[9]	4·6	0·4	2·4

EBS, EB simplex; JEB, junctional EB; DEB, dystrophic EB. [a]Point prevalences in Scotland on 1 January 2001. Figures based on Scottish population size in June 2000 of 5 114 600 (data supplied by the General Register Office for Scotland).

known to the medical profession and are readily included in EB registers but, unless they are actively sought, sufferers with mild disease may not come to the attention of researchers. Assessments of the epidemiology of EB in other countries[2–9] suggest incomplete sampling, pronounced regional variation or differing genetic susceptibilities (Table 1). Although the ambitious U.S. national EB registry has so far published valuable information on the largest EB cohort,[9,10] the large size of the American population hinders complete sampling, thus resulting in a tendency to assess only more severely affected EB sufferers. The smaller Scottish population has been actively searched for EB sufferers, resulting in compilation of the most complete EB register of any published to date.

Details of the clinical features of EB simplex and the prevalence and incidence of EB in Scotland have been published previously.[11,12] Between 1960 and 1999, the incidence in Scotland of EB simplex and of DEB was 34·3 and 26·0 per million, respectively i.e. 103 cases of EB simplex and 78 of DEB out of 2 995 052 live births during this period (data supplied by the General Register Office for Scotland). We now present data relating to the clinical features of DEB.

Patients and methods

Since 1992, 128 Scottish individuals have been identified by the authors as suffering from DEB. Methods of identification and assessment of patients have been published previously.[11] Diagnosis was made on the basis of clinical features, family history and, in cases of doubt, by antibody mapping and ultrastructural examination using the recently published revised classification system of Fine *et al.*[10] Dominant DEB (DDEB) was diagnosed when typical clinical features were present in two or more individuals spanning at least two generations. All patients with severe clinical features, and many with milder disease, underwent skin biopsy and ultrastructural assessment. Nine patients with very mild disease and lacking a family history did not have skin biopsies and were classified as having DEB of uncertain inheritance (DEB-unc). DNA from selected patients was analysed (Dr J.McGrath) for the presence of *COL7A1* mutations. We continue to record newly diagnosed EB sufferers, both those referred to our department and those diagnosed at other Scottish hospitals.

The authors have examined 97 DEB sufferers from 50 families on at least one occasion and systematically recorded their clinical findings at initial presentation. Of the remaining 31 patients, 20, who have not been clinically assessed, were identified by affected relatives in whom the diagnosis of DEB had been confirmed. Nine were diagnosed at the Royal Infirmary of Edinburgh as suffering from DEB. However, clinical information is incomplete, and none is available for the two patients diagnosed at other Scottish hospitals—one an adult suffering from DDEB and the second a neonate suffering from recessive DEB (RDEB-loc) of Hallopeau–Siemens subtype (RDEB-HS). This study reports the clinical findings at initial assessment of the 97 patients seen by the authors.

Results

Four clinical variants of DEB were identified. DDEB occurred in 88 patients (68%). Twenty-four patients (19%) had no family history of DEB and clinical features of mild to moderate severity (DEB-unc). On the basis of individual pedigrees, recessive inheritance was judged to have occurred in 16 patients (13%): nine (7%) with RDEB-HS and seven non-Hallopeau–Siemens RDEB (RDEB-HS).

Dominant dystrophic epidermolysis bullosa

Detailed clinical information is available for 64 of the 88 individuals from 18 families. Subjects were aged between 2 days and 76 years at interview (mean 27 years). Thirty-six were female. Age at onset of blistering was known in 43 of the 64 patients and varied between birth and 5 years (Table 2). Clinical features are summarized in Table 3. With the exception of seven children, four of whom were under 1 year old, scars were present over bony prominences in all

Table 2. Age at onset of blisters in dystrophic epidermolysis bullosa (DEB)

Age at first blister	RDEB-HS $n = 8$	RDEB-loc $n = 4$	RDEB-inv $n = 2$	DDEB $n = 64$	DEB-unc $n = 19$	Total
Birth	7	3	1	6	4	21
1–7 days	1	0	0	12	5	18
1–4 weeks	0	1	1	0	2	4
1–6 months	0	0	0	11	6	17
7–24 months	0	0	0	10	0	10
2–5 years	0	0	0	4	0	4
Unknown	0	0	0	21	2	23

RDEB-HS, recessive DEB of Hallopeau–Siemens subtype; RDEB-loc, localized RDEB; RDEB-inv, inverse form of RDEB; DDEB, dominant DEB; DEB-unc, DEB of uncertain inheritance.

Table 3. Clinical features of dominant dystrophic epidermolysis bullosa in adults and children

	Children aged 12 years or under ($n = 25$), %	Adults ($n = 39$), %
Albopapuloid lesions	8	49
Blisters	84	64
Scars	72	100
Milia	44	15
Contractures	0	4
Nail loss/dystrophy	44	85
Otitis externa	8	8
Constipation	12	21
Anal fissures	12	21
Dysphagia	4	23
Oral blisters	28	36
Dental disease	8	51
Microstomia	0	5

patients. Milia were noted in 17 subjects (27%), both adults and children. Nail dystrophy, present in 44 patients (69%), was not seen below the age of 3 years. Of the 22 individuals (34%) with significant dental abnormalities, i.e. excessive dental caries or its sequelae, three were under 20 years of age and 13 reported oral blistering. A further nine patients with oral blisters had normal dentition. Only two of the 12 (19%) subjects with anal fissures were children, the youngest being 2 years old.

Nine adults and one child aged 9 years (16%) complained of dysphagia. All but one also had anal fissures (seven patients) and/or constipation (five patients). A further six patients complained of troublesome constipation and two had some restriction of mouth opening. A recent barium examination in one adult with long-standing dysphagia and who intermittently produced oesophageal casts, did not reveal any abnormality. Although pseudosyndactyly was not observed in any individuals, flexion contractures of the fingers were seen in four adults (6%). No patients had eye involvement. Two adults (3%) had itchy pretibial plaques, and 'EB pruriginosa' and albopapuloid lesions were apparent in 23 (36%) patients. Two heterozygous glycine substitution mutations of *COL7A1* (one reported previously[13]) were detected in patients from three families.

Dystrophic epidermolysis bullosa of uncertain inheritance

Clinical information is available for 19 unrelated subjects. One man with mild DEB limited to the skin and oral mucosa was the cousin of a patient with RDEB-HS who died before analysis of *COL7A1* mutations was available.[14] The remaining patients in this group had no family history of DEB. Ages at interview ranged from 6 months to 39 years (mean 13·9 years). Nine (47%) had mild DEB while the remainder had more troublesome DEB of varying severity. All had scars, 13 (63%) had milia and 11 (58%) had nail loss or dystrophy. The youngest child to have abnormal nails was 21 months old. She was the only individual in this group born with a localized area of absent skin ('Bart's syndrome') and the only one to suffer from recurrent corneal erosions. Pseudosyndactyly was not observed but mild flexion contractures of the fingers were seen in one adult. Nine subjects (47%) had albopapuloid lesions and two (11%) had EB pruriginosa. Both individuals with this clinical variant of DEB also had involvement of the external ear. Ten patients (53%) experienced oral blisters. Dental disease was present in five (26%), all but one of whom suffered oral blistering. One adult with normal dentition had both restriction of mouth opening and limitation of tongue protrusion (ankyloglossia). Anal fissures occurred in five individuals (26%), both adults and children, and were associated with constipation in four. A further three subjects also complained of constipation. Oesophageal strictures were demonstrated in two of the four patients (21%) who experienced dysphagia, which in a third

was associated with the regular production of oesophageal casts.

Recessive dystrophic epidermolysis bullosa of Hallopeau–Siemens subtype

All nine individuals in this group were unrelated and had unaffected parents. One had a cousin suffering from mild localized DEB-unc: details of this family have been reported previously.[14] The parents of one child were cousins but there was no evidence of consanguinity in the other families. Clinical information is available for eight patients who were aged between 3 days and 34 years at interview. Three were less than 1 year old, two were children aged 3 and 10 years and three were adults aged 20, 23 and 34 years. Blisters were present at birth in all but one, in whom they appeared during the following 24 h. Two neonates also had congenital localized areas of absent skin. Although not a prominent feature, milia were visible in all patients and scars were seen on all but the youngest infant. Some features typical of RDEB-HS, including dysphagia, dental disease and the consequences of scarring (i.e. flexion contractures of the limbs, microstomia, ankyloglossia and mitten deformity of the hands), were age related, being present in all adults and the elder but not the younger child nor the infants. Mild flexion contractures of the fingers and some loss of interdigital web spaces were present in the 3-year-old child. All subjects, including the infants, had nail loss or dystrophy. Each adult had very sparse scalp hair and none had experienced puberty. Involvement of the external ear was seen in three patients. Five subjects, infants and adults, experienced corneal erosions and one adult had ectropion. Constipation proved troublesome in six patients, being associated in three with anal fissures. Only one subject, an infant, did not have oral blisters. Neither EB pruriginosa nor albopapuloid lesions were observed. Barium studies in two adults confirmed the presence of oesophageal strictures. Analysis of DNA from one patient identified compound heterozygous mutations of *COL7A1*. Since the onset of this study, the effects of scarring have become more apparent in the infants and dysphagia has become troublesome to such an extent that two have now received gastrostomies. Two adults died during the study period—one from metastatic squamous cell carcinoma (SCC) arising in the skin and the second from aspiration of gastric contents during an influenza-like illness. The eldest adult has recently developed her first cutaneous SCC.

Non-Hallopeau–Siemens recessive dystrophic epidermolysis bullosa

Localized recessive dystrophic epidermolysis bullosa. In five adults, the clinical features were relatively mild and the pedigrees suggestive of recessive inheritance. Four were siblings and have been previously reported.[15] There was no evidence of consanguinity in either family. Clinical information is available for three of the four siblings and for the unrelated patient. Blisters had been present at birth in three patients and had appeared during the following 4 weeks in one; there had been congenital absence of a localized area of skin in three. All blistered readily in response to minor trauma during childhood and teenage years, but reported marked improvement of skin fragility during adult life. When interviewed for this study the four subjects were aged between 36 and 47 years. They had all experienced normal puberty and had unaffected children. All had atrophic scars over bony prominences and either nail loss or dystrophy. Milia, mild finger flexion contractures, albopapuloid lesions and external ear involvement were each seen in individual patients. Pseudosyndactyly and corneal erosions did not occur. Two sisters experienced pruritus, which in the first was accompanied by pruriginous change over the pretibial areas and posterior shoulders and in the second affected the perineum. A number of contact sensitivities had been identified by the referring hospital in this latter patient. Oral blisters, dental disease and anal fissures were present in every patient and constipation was troublesome in three of the four. Two had some restriction of mouth opening and protrusion of the tongue. Oesophagoscopy and barium swallows had previously revealed the presence of oesophageal webs in all four and, in addition, oesophageal strictures had been seen in two.[5] The siblings had required repeated oesophageal dilatations from the late teenage or early adult years, but after 10 years, dysphagia resolved in two, at the age of 26 and 36 years.

Inverse form of recessive dystrophic epidermolysis bullosa. Two unrelated woman aged 48 and 57 years at interview were affected by flexural erosions in addition to blisters and scarring over bony prominences. The parents of the elder, an only child, shared a common ancestor from a remote part of Scotland. Milia, finger flexion contractures, loss of nails and oral blisters were seen in both patients. They also both had dental disease, ankyloglossia, mild microstomia and anal fissures. Constipation was troublesome only in one.

Both patients experienced dysphagia as a consequence of oesophageal strictures, and underwent repeated oesophageal dilatations. Both also suffered blistering of the pinnae and one had bilateral stenosis of the external auditory meatus associated with deafness. The elder of the two patients died during the study from an SCC arising in the oesophagus.

Albopapuloid lesions

In a significant minority of patients with DDEB (20 patients, i.e. 31%, from 10 families), examination of the lumbar area revealed multiple slightly depressed areas resembling subtle atrophic scars, each measuring about 1 cm in width and having a finely wrinkled surface (Fig. 1).

These were found in DDEB of all severities but were not universally present in adult members of any pedigree. They were also seen in DEB-unc (eight unrelated patients) and in one patient with localized RDEB (RDEB-loc). The youngest subject in whom they were present was 6 years old.

In some cases, albopapuloid lesions were represented by small ivory-white papules[16] (Fig. 2), which occurred on the upper trunk, arms and buttocks of six patients (two with mild DEB-unc and four unrelated individuals suffering from DDEB of a wide range of severity). The youngest affected was 14 years old. Two patients complained of associated itching. Both variants of albopapuloid lesions were visible simultaneously on the skin of two individuals.

'Epidermolysis bullosa pruriginosa'

Intense itching, associated with the formation of pretibial lichenified nodules or plaques, is a distinctive clinical picture that is genetically diverse.[17,18] In our study it was seen in DDEB, RDEB and DEB-unc. The appearance may be confused clinically with both hypertrophic lichen planus and nodular prurigo. Among the Scottish families, itchy pretibial lesions were present in two unrelated adults with DDEB and two with DEB-unc. In each of these latter patients, the lesions were remarkably extensive and linear (Fig. 3). Onset of hypertrophic scarring was delayed until adult life.

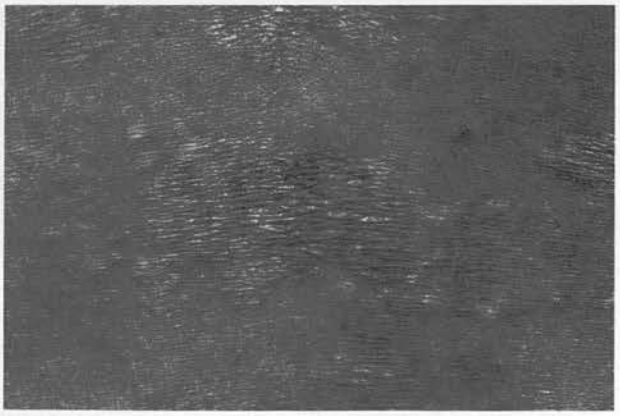

Figure 1. Albopapuloid lesion on the lumbar area.

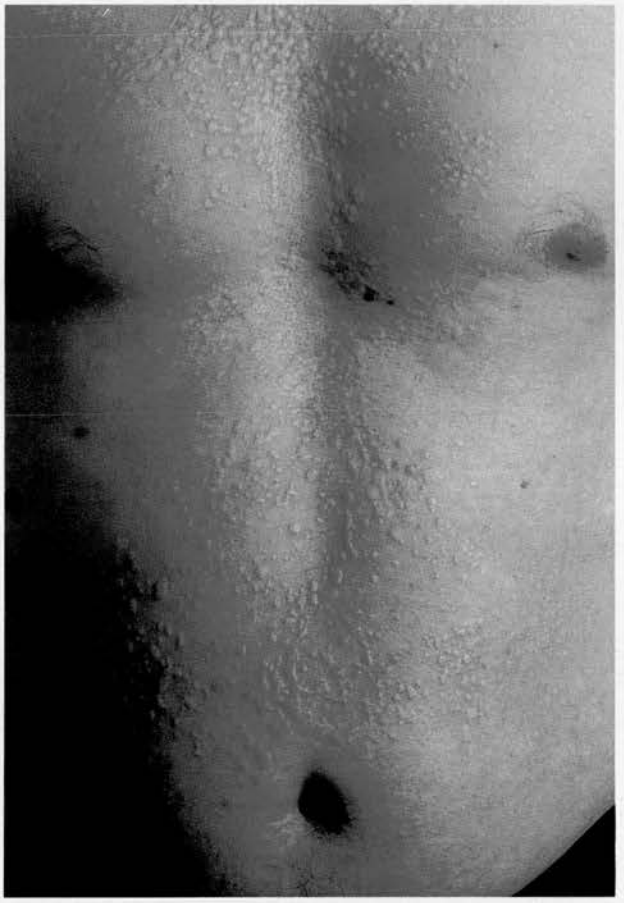

Figure 2. Albopapuloid lesions on the trunk.

Perineal itching in DEB has been reported previously,[19] and was seen in one Scottish adult woman with RDEB-loc. Her affected sister also experienced itching but the clinical appearance in her case was that of nodular prurigo.

Discussion

By actively seeking EB sufferers within a population we have found that the prevalence of DEB in Scotland (now 24 per million) far exceeds any previous reports. The high Scottish prevalence of DDEB, although

Figure 3. Hypertrophic linear plaques on pretibial skin in epidermolysis bullosa pruriginosa.

undoubtedly an underestimate, is particularly striking when compared with data from the U.S.A.[9] (Table 4) where it is likely that the large population and different health care system may hinder full detection of EB sufferers. Despite these apparent differences, our clinical findings are broadly in agreement with those of the U.S. national EB registry. Milia, a non-specific feature of most blistering disorders, were less common in Scottish DDEB sufferers, perhaps reflecting inclusion in the U.K. EB register of patients with mild disease who were not actively blistering at the time of clinical examination. Otitis externa was found more frequently in all subtypes of DEB in Scotland, but the difference was particularly marked in those with RDEB-HS (37% in Scotland, 9% in the U.S.A.) and RDEB-loc (25% in Scotland and 4·1% in the U.S.A.). Other clinical features (corneal erosions, nail involvement, scarring, contractures, pseudosyndactyly, constipation, dysphagia, oral blistering, ankyloglossia and microstomia) occurred with similar frequencies to those reported from the U.S.A.[9,10]

With the exception of one individual, all Scottish RDEB-HS sufferers had blisters at birth, the remaining patient developing blisters within the following 24 h. However, early blistering does not inevitably indicate either recessive inheritance or a poor prognosis. Eighteen of the 43 DDEB patients in whom the age at onset of blistering is known, started to blister within the first week postpartum (Table 2). Although eight (44%) of this early onset group experienced a variety of troublesome extracutaneous features, the remaining 10 (56%) had mild disease confined to the skin. At least 37 (58%) Scottish DDEB sufferers had no blisters at birth, and 25 (39%) did not develop blisters until after the fourth week of life. This finding contrasts with the recently published revised classification of EB, which states that in DDEB it is usual for blisters to be present at birth.[10] This apparent discrepancy probably reflects more complete sampling of Scottish DDEB patients and inclusion of more patients with mild disease.

For most sufferers, DDEB does not impose serious difficulties, and many experience improvement of the blistering tendency during early adult life; some even consider their skin to be normal. Consequently, there is considerable under-reporting leading to the misleading impression that DEB is inevitably a severe disorder. For many the cosmetic consequences of scarring are the most troublesome aspect of their disease, causing psychological difficulties and social embarrassment rather than physical disability. A significant minority of DDEB sufferers, in both Scotland and the U.S.A.,[9] have a more severe phenotype, experiencing contractures, microstomia, dysphagia and anal fissures, clinical features that are more often associated with RDEB. Gastrointestinal manifestations of DEB can occur even

Table 4. Prevalences of dystrophic epidermolysis bullosa (DEB) subtypes per million in the U.S.A. and Scotland

	DDEB (*n*)	DEB-unc (*n*)	All RDEB (*n*)	RDEB-HS (*n*)	RDEB-nHS (*n*)
Scotland	17·2 (88)	4·7 (24)	2·54 (13)	1·36 (7)	1·17 (6)
U.S.A.	0·87 (216)	0·37 (91)	0·8 (98)	0·36 (90)	0·43 (108)

DDEB, dominant DEB; DEB-unc, DEB of uncertain inheritance; RDEB, recessive DEB; RDEB-HS, RDEB of Hallopeau–Siemens subtype; RDEB-nHS, non-Hallopeau–Siemens RDEB.

in the absence of active blistering and, unless cutaneous signs of DDEB are sought in such patients, EB may not be apparent as the cause of anal disease or dysphagia. In both Scotland and the U.S.A., dysphagia is apparently more common than oesophageal webs or strictures. Not all our patients underwent barium studies, but it is significant that in some with troublesome dysphagia and a history of producing oesophageal casts, no structural abnormality could be demonstrated.

Because scarring and its sequelae become more apparent with increasing age, some of the hallmarks of DEB may not be found in infants or children. The mitten deformity, a characteristic feature of RDEB-HS, was not seen in any child aged under 4 years, although finger flexion contractures and some loss of digital web spaces were apparent by the age of 3 years. Nail loss or dystrophy, one of the most common physical signs in DEB, was absent in those DDEB sufferers under 3 years old and occurred in only 15% of children with this subtype aged 5 years or under (data not shown). Contractures, uncommon in DDEB, occurred solely in adults, and dysphagia was present in only one child with this subtype.

Dental disease due to caries occurs in all subtypes of DEB and is preventable. The teeth are structurally normal,[20] but restricted access as a result of microstomia and pain from oral blisters contributes to poor oral hygiene. In those suffering from dysphagia, a high carbohydrate diet may also contribute to dental decay. Of those with DDEB in this study, none of the 15 children under 5 years old and only three subjects under 20 years old had evidence of dental disease.

Albopapuloid lesions (Figs 1 and 2) are a striking clinical finding in DEB, but they do not appear to be of diagnostic or prognostic significance. Although most common in DDEB, they also occur in other subtypes of DEB and have been reported in junctional EB.[21]

As yet, there is no explanation for the development of the EB pruriginosa phenotype. Molecular studies have revealed a range of both heterozygous and compound heterozygous *COL7A1* mutations in this distinctive clinical variant.[18] Identical mutations have been found in DEB sufferers with a non-pruriginous phenotype, indicating that additional genetic or environmental factors, perhaps atopy, may be implicated.[18]

The reason for improvement of the blistering tendency or dysphagia with age is not known. Phenotypic variation within individual pedigrees is also difficult to explain. Analysis of *COL7A1* mutations or of genes encoding structurally or functionally related proteins may provide some of the answers. When these investigations become more readily accessible, accuracy of diagnosis of EB subtype will be improved. Like previous studies of EB, we have demonstrated overlap of clinical features between the subtypes of DEB. It is important that diagnosis of EB subtype should not rely on clinical features alone but should be aided by ultrastructural studies, antibody mapping and, where appropriate, by mutational analysis. Determination of *COL7A1* mutations is essential if accurate genetic counselling is to be given to families at risk of having a severely affected child and to those patients with moderately severe DEB-unc. These latter patients usually prove to have mild RDEB or, less commonly, *de novo* sporadic dominant mutations. More unusual inheritance patterns such as uniparental disomy have been reported,[22] with profound implications when assessing risks to future pregnancies.

Challenges for the future[23] include preventing the devastating effects of scarring both in the small proportion of severely affected DEB sufferers and in those with milder disease for whom scars are a cosmetic embarrassment. Prevention and better treatment of SCCs, the major cause of death in RDEB,[10] are of great importance, and frequent total skin inspections are an essential part of the management of those with severe DEB. Although DEB can be a devastating disorder, presenting numerous and difficult challenges to patients, clinicians and researchers, for most DEB sufferers it is not a severe disease.

Acknowledgments

We gratefully acknowledge the financial support of DEBRA U.K. and thank Scottish dermatologists for their help in maintaining the EB register.

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Quality of life in epidermolysis bullosa

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Summary

The quality of life of people with epidermolysis bullosa (EB) living in Scotland was assessed by postal questionnaire using the Dermatology Life Quality Index (DLQI) and the Children's Dermatology Life Quality Index (CDLQI). There were 143 people with EB simplex (EBS) and 99 individuals with non-Hallopeau–Siemens subtypes of dystrophic EB (DEB). A further six individuals had the severe Hallopeau–Siemens subtype of DEB (RDEB-HS). The overall response was 48% (EBS 52%, DEB 40% and RDEB-HS 83%). Impairment of quality of life (QOL) was greatest in those with RDEB-HS, mean scores (adults, 18; children, 22) exceeding those of any skin disorder previously assessed. The effect on QOL of EBS and other subtypes of DEB was similar to that of moderately severe psoriasis and eczema. EBS had a greater impact on QOL than the non-Hallopeau–Siemens subtypes of DEB (EBS adults mean score, 10.7; EBS children mean score, 15; DEB adults mean score, 7.5; DEB children mean score, 11.5).

Introduction

Skin disease can have a profound impact on many aspects of daily life. Pain, itch, odour and time-consuming dressings are problems which impinge on self confidence, social activities personal relationships, education and employment. The Dermatology Life Quality Index (DLQI)[1] (Fig. 1) and the Children's Dermatology Life Quality Index (CDLQI)[2] (Fig. 2) have emerged as useful tools for assessing the quality of life of those with skin diseases, comparing severities of different disorders and judging the effectiveness of treatments.[1–10] Our aim was to document the impact of variants of epidermolysis bullosa (EB) on quality of life.

Methods

Details of all EB sufferers living in Scotland were derived from the EB Register.[11] In the first of three diagnostic groups (group A), there were 143 EB simplex (EBS) sufferers. One-hundred and eleven adults and 27 children had either EBS Weber-Cockayne or EBS-Köbner and five patients (three adults and two children) had the Dowling-Meara subtype of EBS (EBS-DM). Adults were aged between 16 and 86 years (mean 41 years) and children between 1 and 15 years (mean 10 years). In group B there were 99 dystrophic EB (DEB) sufferers comprising 53 adults and 20 children with dominant DEB, 12 adults and eight children with DEB of uncertain inheritance (DEB-unc) and six adults with non-Hallopeau–Siemens recessively inherited DEB (RDEB-nHS). Adults were aged between 16 and 84 years (mean 44 years) and the ages of the children ranged from 3.5 to 15 years (mean 10 years). Six patients with RDEB-HS were considered in group C, an adult aged 40 years and five children aged from 3.5 to 15 years (mean 6 years, median 3.5 years).

The DLQI and an explanatory letter stressing the anonymity of replies were posted in early May to every Scottish EB sufferer aged 16 years or over. Parents of those aged under 16 were sent a copy of the CDLQI for each of their affected children. After an interval of 2 weeks, a reminder was posted to every patient. To enable correlation of replies with subtype of EB, questionnaires were colour coded, different colours corresponding to each of the three diagnostic groups.

The (C)DLQI contains 10 questions relating to experiences during the previous week.[1,2] Each question has five possible answers (very much, a lot, a little, not at all or not relevant) which score 3, 2, 1, 0 or 0,

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Accepted for publication 12 June 2002

1 Over the last week, how itchy, sore, painful or stinging has your skin been?

2 Over the last week, how embarrassed or self conscious have you been because of your skin?

3 Over the last week, how much has your skin interfered with you going shopping, or looking after your home or garden?

4 Over the last week, how much has your skin influenced the clothes you wear?

5 Over the last week, how much has your skin affected any social or leisure activities?

6 Over the last week, how much has your skin made it difficult for you to do any sport?

7 Over the last week, has your skin prevented you from working or studying?

If "No", over the last week, how much has your skin been a problem at work or studying?

8 Over the last week, how much has your skin created problems with your partner or any of your close friends or relatives?

9 Over the last week, how much has your skin caused any sexual difficulties?

10 Over the last week, how much of a problem has the treatment of your skin been, for example by making your home messy or by taking up time?

Figure 1 The DLQI.

1. Over the last week, how itchy, "scratchy", sore or painful has your skin been?

2. Over the last week, how embarrassed or self conscious, upset or sad have you been because of your skin?

3. Over the last week, how much has your skin affected your friendships?

4. Over the last week, how much have you changed or worn different or special clothes/ shoes because of your skin?

5. Over the last week, how much has your skin trouble affected going out, playing or doing hobbies?

6. Over the last week, how much have you avoided swimming or other sports because of your skin trouble?

7. Last week, was it either : school ☐ — If school time: over the last week, how much did your skin affect your school work?

   or (answer only one) holiday time ☐ — If holiday time: how much over the last week, has your skin interfered with your holiday plans?

8. Over the last week, how much trouble have you had because of your skin trouble, with other people calling you names, teasing, bullying, asking questions or avoiding you?

9. Over the last week, how much has your sleep been affected by your skin problem?

10. Over the last week, how much of a problem has the treatment of your skin been?

Figure 2 The CDLQI.

respectively. Patients are asked to tick one answer for each question.

Results

Replies were received from 75 patients in group A (57 adults and 18 children), a response of 52% (adults 50%, children 62%). In group B, there were 32 replies from adults (45%) but only eight replies (29%) from children, an overall response of 40%. Only one of the patients in group C, a child, did not reply (83% response). Overall, the response was 48%.

Adults – EBS

The mean total score in group A was 10.7 (range, 0–26; median, 10). Question 1 (which enquired about symptoms) and question 6 (relating to sport) had the highest mean scores (1.6 and 1.5; median, 2 in each case) (Fig. 3). The lowest mean score (0.3) was for question 9 which asked about sexual difficulties. Four patients aged

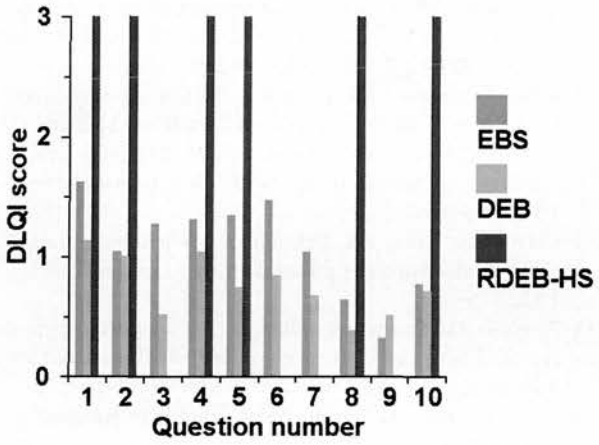

Figure 3 Mean DLQI scores in adult EB patients.

between 23 and 39 each scored a total of zero. The question answered 'very much' most often (20 patients, 35%) was number 6.

Adults – DEB

The mean total score was 7.5 (median, 4; range, 0–28). Questions 1, 2 and 4 (symptoms, embarrassment and clothing) achieved the highest mean scores of 1.2, 1.0 and 1.0 (median scores of 1 in each case) and question 8 (relationships) had the lowest mean score (0.4; median, 0) (Fig. 3). Like EBS, the question awarded 'very much' by the greatest number of patients was number 6 (sport), which was selected by seven (23%) patients. Five patients, three aged between 67 and 84 and two of unknown age, recorded total scores of zero.

Children – EBS

Children with EBS recorded a mean total score of 15 (median 15, range 0–30). Question 1 achieved the highest mean score (2.1, median 2), followed by question 5 (play and hobbies; mean, 1.9; median, 2) (Fig. 4). The lowest score was for question 8 (teasing and bullying; mean 0.9, median 0.5). Only one patient (aged 8.5 years) recorded a total score of 0. In keeping with adult responses, the question awarded 'very much' by the greatest number of children (nine children, 50%) was number 6 (sport).

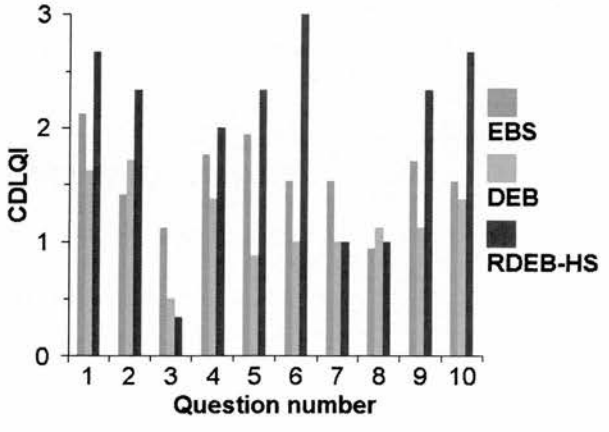

Figure 4 Mean CDLQI scores for children with EB.

Children – DEB

The mean total CDLQI score for children with DEB was 11.5 (median, 12.5; range, 0–23). Question 2 (embarrassment and sadness) recorded the highest mean score (1.7; median, 2), followed by question 1 (symptoms; mean, 1.6; median, 2) (Fig. 4). The lowest mean score was for question 3, which asked about friendships (mean, 0.5; median, 0). Question number 1 was awarded the maximum score by the most patients (three patients, 37.5%). One child, aged 8 years, scored a total of zero.

RDEB-HS

The one adult with RDEB-HS recorded a total score of 18. Question numbers 3 (household chores), 6 (sport), 7 (work), and 9 (sexual difficulties) were each awarded a score of 0, remaining questions scoring 3.

The four children with RDEB-HS scored a mean total of 22.0 (median, 21; range, 17–29). All patients answered 'very much' to question 6 (sport). Question 3 (friendships) recorded the lowest mean score (mean, 1.0; median, 1).

Discussion

Despite the limitations of a postal survey and the possibility of a biased response, our results reflect the wide variety of problems encountered by EB sufferers. Adults and children with both EBS and DEB experience an impairment of quality of life (QOL) equivalent to that caused by moderate to severe psoriasis and atopic

Table 1 Mean (C)DLQI scores in skin disorders.

	Adults	Children
Atopic eczema	4.14[3]–16.2[4]	7.7[2]–12.7[5]
Psoriasis	4.5[3]–13.9[4]	–
Hidradenitis suppurativa	8.9[6]	–
Urticaria	7.5–15[7]	–
Behçet's syndrome	5.7[8]	–
RDEB-HS	18.0	22.0
EBS	10.7	15
DEB	7.5	11.5

eczema (Table 1). Like most other skin disorders, question 1, relating to symptoms, was scored most highly by those in groups A and B. Comparison with other studies suggests that RDEB-HS causes greater impairment of QOL than any skin disease previously assessed (Table 1). This impairment is further emphasized by the fact that scores from some of these patients were artificially lowered by responses 'not at all' or 'not relevant' to questions on matters entirely incompatible with the lifestyle of RDEB-HS sufferers. These include sport, sexual difficulties, and domestic duties.

EBS is often considered to be the mildest subtype of EB but this survey indicates that it has a more marked effect on QOL than the non-Hallopeau–Siemens variants of DEB. Patients' replies reflect the greater restriction of physical, social and sporting activities caused by EBS compared to DEB and also its greater impact on employment and education. Despite the often visible lesions of DEB, children with EBS, whose blisters are usually concealed by footwear, find more difficulties with friendships. Pain prevents participation by those with EBS in the more physical aspects of normal childhood and in our experience, this can lead to social isolation or psychological problems. Undoubtedly, some DEB sufferers do experience great difficulties, but the majority of those with dominant DEB and even some with RDEB-nHS, have only mild disease.[13]

Although some patients with EBS and DEB experience an improvement of the blistering tendency during adult life, many do not[12] and for them impairment of QOL is lifelong.

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